THE MECHANISM OF TRANSMISSION OF NON-PERSISTENT VIRUSES BY APHIDS.

A thesis presented by

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This thesis contains no material which has been accepted for the award of any other degree or diploma in any University, nor any material previously published or written by another person except when due reference is made in the text.

R. G. Garrett.

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CONTENTS

SUMMARY

While probing plants labelled with phosphorus-32, the aphids Myzus persicae and Hyperomyzus lactucae imbibed tracer into, and ejected it from the cibarium. This uptake of tracer, and therefore sap, is considered to be important in relation to host-recognition and non-persistent virus transmission by aphids. Factors that affected virus transmission (pre-access fasting, duration of access-probe and different aphid species) similarly affected sap-uptake and ejection. Aphids carrying tracer or virus did not always transmit them; however, failure to transmit tracer or virus does not imply that the aphids could not transmit them. When aphids were allowed successive access-probes on two plants infected with different viruses (potato virus Y then cucumber mosaic virus or vice-versa), transmission of the virus acquired in the first probe was delayed when aphids were carrying virus acquired in the second probe.

It is concluded that aphids probably carried transmissible virus within the cibarium. During the subsequent probes, virus could be ejected from the cibarium, or displaced within it by imbibed sap and transmitted during a later probe. Thus, the inoculation probes must be considered also as access-probes and the imbibed sap would gradually reduce the probability of virus transmission for each successive probe.

The widely accepted hypothesis that non-persistent virus is transmissible only when carried at or near the stylet-tips, is insufficient and requires, in addition, a wide range of subsidiary hypotheses which have not been verified experimentally. Among those commonly cited to explain particular properties of the non-persistent virus transmission are:-

(1) Stylets are decontaminated by the hardening salivary sheath; (2) Feeding aphids produce inhibitors but fasting aphids do not; (3) Virus is more readily available in the superficial tissues; (4) Fasting increases transmission rates by allowing aphids to ensheath their stylets in the labial groove; (5) Non-persistence depends on the inactivation of virus at the stylets.

In this thesis an alternative explanation is proposed: that transmissible non-persistent viruses are carried primarily in the cibarium, and most factors affecting transmission do so by their effects on sap imbibition and ejection. That is, the properties of transmission of these viruses depend on aphid behaviour rather than on direct effects on the viruses or their availability to the aphid. This mechanism is sufficient to explain most aspects of the transmission of these viruses in terms of only the probability of sap-uptake and ejection and the amounts involved. The most important factors that can be explained in this way are:-

(1) Pre-access fasting increases transmission rates:

(2) Transmission rates rise and fall as access-probe duration increases;

(3) Some aphid species are more efficient than others in transmitting a wide range of viruses;

(4) Non-persistence of the viruses in their vectors;

(5) In serial transmission experiments, viruses are transmitted intermittently;

(6) Transmission frequencies rarely exceed 0.7.

INTRODUCTION

Transmission of virus by aphids

The non-persistent viruses (Watson & Roberts, 1939) are transmitted by aphids while they probe rather than feed. In contrast to the persistent viruses, they usually survive for only a few hours in their vectors. Optimum transmission rates are obtained with aphids immediately after their access-probes on diseased plants. The subsequent fall in transmission rate with storage between access and inoculation probes is greater for aphids that are stored on plants than those stored in glass vials. On the other hand, persistent viruses cannot usually be transmitted until several hours or days have elapsed between long access and inoculation feeds. Transmission rates then increase to a maximum several days after access-feeds.

The hypothesis of stylet contamination.

Because the non-persistent viruses can be transmitted immediately after brief access-probes and do not survive in moulting aphids, they are generally believed to have a superficial relationship with their vectors. The early work by Watson (1936, 1938) and by Watson & Roberts (1939) did much to elucidate the conditions necessary for transmission of these viruses. However, the mechanism of transmission was little understood.

The effects of pre-access fast and access-probe duration

were explained by proposing that viruses were inactivated by substances produced by aphids when feeding (Watson & Roberts, 1939) but for which there was no direct evidence. The main reason for rejecting the idea that these viruses were transmitted in a mechanical way (Doolittle & Walker, 1928; Hoggan,1933; Osbourne,1937) was the specificity in transmission shown by some aphids and viruses (Watson & Roberts,1939). These examples could sometimes be explained by ranking aphid species in order of general efficiency as vectors, but several anomalies persist (Kassanis, 1947; Kvicala, 1945). To explain the exceptions, the variations between aphid species and the variations between different viruses or virus strains, hypotheses have been proposed that include specific inactivation of viruses by aphid secretions (Watson & Roberts, 1939; Day & Irzykiewicz,1954), differences in aphid behaviour (Day & Irzykiewicz,1954; Sylvester,1954), differences in surface adherence (Van der Want, 1954) and the ability of the salivary sheath to filter virus (Sukhov,1944). Sylvester (1954) suggests that stylet-borne viruses may be transmitted with a plug of saliva carried on the stylet tips, and that specificity depends on the interaction of virus, saliva and host materials, together with variations in the behaviour of different aphid species while they probe plants.

Bradley & Ganong (1955[a b]) and Bradley (1966) were the first to attempt direct methods of locating transmissible non-

persistent virus on aphid stylets. Three of these methods are important. Bradley (1963a) reported that when aphids made access or inoculation probes through 'Parafilm' membranes, they transmitted less often than when they probed plants directly. This was thought to show that transmissible virus was carried externally on the stylets and that the membrane wiped virus from them. Later, Bradley, (1963 b) showed that a paraffin oil in the membrane was probably responsible for the decrease in transmissions. More recently, it has been shown that oils can have a systemic effect on virus transmissions; when applied to one side of a leaf, they can reduce the number of transmissions made by aphids that probe the opposite surface (Külps, 1968). The oils may migrate through the leaf and may affect the physiology of the plant, and perhaps the response of the aphid to it, rather than directly affect the virus on the aphid stylets.

A second technique directed at finding the location of transmissible virus is to treat exposed stylets with chemicals known to inactivate plant viruses. Formaldehyde was found to be particularly effective. Bradley and Ganong (1955b) showed that stylet insertion into 0.03% or stronger solutions of formaldehyde between access and inoculation probes prevented or reduced virus transmission. Treatment of stylets before the access-probe also prevents virus transmission for some time after treatment, but only with formaldehyde concentrations greater than 0.05%

(Sylvester & Bradley, 1962).

Ultra-violet irradiation of exposed stylets has the advantage that its effects are localised (Bradley & Ganong, 1955a) whereas solutions applied to stylet tips may migrate and act at sites distant to the point of application. Irradiation of the stylet tips alone, after access but before inoculation probes, renders most aphids non-viruliferous (Bradley & Ganong, 1955a, 1957 ; Orlob & Bradley,1961; Wade,1962) but irradiation of stylets except the tips does not affect the transmission of virus unless done before the access probe (Bradley & Ganong, 1955a). This led Bradley & Ganong (1955a, 1957) to conclude that transmissible virus was carried only at the stylet tips. However, Bradley (1964) also reports that irradiation of only the stylet tips before the aphids make access-probes, reduces virus transmission for at least 15 min. after treatment.

The various treatments applied to stylets 'inactivate' some critical part of the transmission mechanism. The treatments have invariably been thought to inactivate virus, but this is not necessarily so. The treatments often prevent or reduce virus transmission when applied before the access-probe and so must affect the aphid; in the presence of such effects, their action on virus cannot be detected. Aphid stylets are sensitive to the treatments applied to them, some of which may affect feeding and may even induce premature birth of nymphs (Bradley, 1962).

The treatments do show that the stylet tips (presumably the four dendrites of the mandibulary nerves) influence virus transmission. However, they do not show where on the stylets transmissible virus is carried, nor even whether these viruses are carried on the stylets.

Although recent reviews have usually taken for granted the hypothesis of stylet contamination (Swenson, 1968; Pirone, 1969) it cannot be considered proven. Its popularity must be attributed more to the lack of a suitable alternative than to the evidence on which it is based.

Behaviour of probing and feeding aphids

Although aphids transmit non-persistent virus while probing plants, there is little information on their behaviour during probing other than rates of stylet insertion and the secretion of saliva.

Aphids usually feed on the phloem of their host plants, but it may take 15 min. or more for them to insert their stylets to the phloem (Roberts, 1940; Van Hoof, 1958). Before attempting to feed, aphids usually insert their stylets one or more times into the host. This is characteristic of the behaviour for optimum transmission of non-persistent virus. The later and more prolonged probes result in fewer transmissions, and aphids that have just ceased feeding on the phloem of diseased plants rarely

transmit virus unless they again make brief probes.

Stylet insertion is probably by force, but enzymes secreted with saliva probably play a role. The aphid secretes onto the host surface a drop of saliva through which the stylets are inserted (Van Hoof,1958). The saliva contains two components (Miles, 1960), one of which gels to form the salivary sheath while the other remains fluid. The fluid component probably contains pectinase (Adams & McAllan, 1956, 1958), polyphenol oxidase and phenolic compounds (Miles, 1964) and a dilute solution of the haemolymph (Miles, 1968).

Pectinase probably aids the insertion of stylets by its action on the middle lamella, and aphids that do not secrete pectinase usually insert their stylets through cells (McAllan & Adams, 1961). However, the extent of damage caused to cell walls by intercellular stylet-insertion is not known, and the stylets may make direct contact with the cytoplast. Also, the stylet tips may be inserted intermittently into adjacent cells and so make contact with the cytoplast (McLean & Kinsey,1964). The secretion of saliva may facilitate intercellular stylet insertion but does not preclude the penetration of cell walls. The ultimate insertion of the stylet tips into phloem cells, which must occur to allow phloem-feeding, shows that this is possible. Myzus persicae Sulz., which secretes pectinase with its saliva (Adams & McAllan, 1956), has been variously reported to probe

between or through cells, or in both ways. Clearly, different populations of the same species may behave in quite different ways.

The way that aphids locate the phloem is not known, and they may do so by accident (Van Emden, Eastop, Hughes & Way, 1969). Subsequent feeding probably depends on sap pressure forcing materials into the aphid (Kennedy, 1950; Van Soest & Meester Manger Cats, 1957). The extent to which the cibarial-pharangeal pump is used during long feeds is not known. The aphid does have the ability to regulate its intake of sap (Kennedy & Stroyan, 1959) and this could be by internal pressure or by the action of the cibarial-pharangeal pump. An oesophageal valve, present at the entrance to the stomach, blocks the aperture when the stomach is full (Martini, 1958). This would seem more likely to prevent back-flow of ingested material from the stomach (Weber, 1930) than sap-flow into it, and would be unable to control materials entering or leaving the fore-gut.

Aphids will not persist in attempts to feed on non-host plants. Wensler (1960) showed that Brevicoryne brassicae (L.) and Aphis fabae Scop. could rapidly recognise host-plants when probing. On hosts, aphids probed briefly then settled to feed. They were attracted to direct green light. However, on non-host plants, aphids probed briefly, became restless and were

repelled by direct green light. She also found that a mustard-oil probably played a part in the recognition of brassicas by B. brassicae. This aphid species normally feeds or breeds only on brassicas but will feed on bean plants (Vicia faba L.) that have been sprayed with sinigrin. The response to the plants was dependent on probing, and the same effects were obtained when aphids probed through collodion films into leaves.

Because aphid behaviour changes rapidly during probing and afterwards, it may be that they acquire stimuli which persist for some time and affect, not only feeding, but also their response to environment. Such stimuli could arise by sap-uptake and the tasting of sap-samples in the epi-pharangeal ganglion. Alternatively, aphid stylets may be sufficiently sensitive to recognise the physical or chemical environments into which they are inserted.

Sap uptake by probing aphids

The recent development of artificial diets for aphids (Auclair & Cartier, 1963; Mittler & Dadd, 1962) was based extensively on the ability of aphids to discriminate between solutions they probed. Such discrimination between solutions may be gustatory (Mittler & Dadd, 1964) and may depend on imbibed sap samples. Although the central cavity of each

mandibulary stylet contains a nerve cell (Forbes,1966) it is likely that four dendrites could only recognise grossly unfavourable substrates such as strong acids (Bradley,1962; Marek,1961). However, uptake has not been clearly demonstrated for aphids probing plants. Day & Irzykiewicz (1953) reported that tracer uptake after the first 10 min. of probing is a linear function of time, but little or no uptake occurs during shorter probes. Similar results were obtained by Watson & Nixon (1953), but some aphids carried small amounts of the tracer after probes lasting from 5 to 10 min. However, these aphids could transfer the tracer to moist filter paper on which they walked and were considered to be superficially contaminated. Since Watson & Nixon labelled leaves by imersing them in tracer, this would seem likely. Ehrhardt (1961) and Hennig (1963) also failed to detect tracer uptake by probing aphids. Hennig's conclusion must be considered reasonable: that if sap-uptake occurs during probing then it plays no significant part in nutrition.

There is evidence that aphids may imbibe sap from parenchyma cells. McLean & Kinsey (1964) correlated changes in potential difference between aphid and leaf with the location of stylets in the host at the end of the expetiment. Patterns of change in flow of electric current were interpreted as caused by either sap-uptake or secretion of saliva. Those

interpreted as caused by sap-uptake were normally associated with aphids that had probed to the phloem. Some aphids had inserted their stylets into parenchyma cells which did not contain a cytoplast and it was concluded that they had imbibed sap.

Probably, aphids do not need phloem pressure for sap-uptake. Recent techniques have allowed aphids to be grown through several generations on chemically defined diets without the need for pressure to aid sap-uptake (Mittler & Dadd, 1962; Auclair & Cartier, 1963). Miles (1969) has observed saliva and water flowing into and from the food canals of non-feeding aphids and it must be concluded that some aphids can suck up materials and eject them, possibly by the action of the cibarial-pharangeal pump. The question arises: do they do so while probing?

The work undertaken for this thesis was designed to assess the importance of sap-uptake by probing aphids, to determine the conditions under which it occurs, and to find out whether it could form the basis of an alternative explanation of non-persistent virus transmission.

MATERIALS AND METHODS

Aphid cultures

The aphid species used in this study were reared on various plants (Table 1, p. 15). For use in experiments, feeding aphids were induced to walk and were aspirated into glass vials stoppered with rubber bungs. When necessary, aphids were stored in these tubes in darkness for pre-access fasting.

Virus cultures

Potato virus Y (PVY) was obtained from the Victorian Plant Research Institute, Burnley, Victoria, and was maintained by sap and aphid transmission to Nicotiana tabacum L. and Physalis floridana

Cucumber mosaic virus (QCMV) was an isolate obtained from Capsicum in Queensland (Francki et al., 1966) and had been subcultured several times by aphid transmission. This raised the frequency of transmission by M. persicae from about 0.3 to 0.65 as shown in table 2 (p. 16). QCMV was maintained in Cucumis sativa L. by aphid transmission.

Infected plants were used for aphid transmissions 10 to 21 days after inoculation. This was normally 4 to 7 days after the appearance of symptoms; the first leaf to show symptoms was used for access-probes.

Table 1. Plant species used for aphid culture.

Aphid species.	Host plant.
Aphis craccivora Koch	V. faba
Aphis gossypii Glover	Cucumis sativa L.
Hyperomyzus lactucae (L.)	Sonchus oleraceus L.
Macrosiphon euphorbiae (Thom.)	S. oleraceus. Datura stramonium L.
Myzus persicae (Sulz.)	D. stramonium Brassica chinensis (Lour.) Rupr.

Table 2. The effect of method of subculture on the transmissibility of QCMV by M. persicae.

Method of subculture:	Manual		Aphids		Chi-square	P
Transmission by aphids:	+	-	+	-		
Initial isolate (a)	15	34				
After subculture 1	18	34	22	26	1.35	> 0.2
After subculture 2	17	25	30	23	2.37	> 0.1
After subculture 3	18	29	34	19	6.19	< 0.02
Total (b)	53	88	86	68	8.27(c)	< 0.01
Chi-square (d)	0.29		3.44			
P	> 0.9		> 0.1			

Footnotes.

(a) previously maintained by sap-inoculation to cucumber.

(b) excluding the original isolate

(c) for treatment totals (1 df)

(d) for effect of subculture within methods, excluding the initial isolate, with 2 df.

Labelling plants with tracer

Radioactive materials were obtained from the A.A.E.C., Lucas Heights, Sydney. Phosphorus-32 was obtained as a solution of orthophosphate in dilute HCl; sulphur-35 was in sulphate form, also in dilute HCl. Concentrations were usually between 5 and 10 mci/ml.

Unless otherwise stated, cucumber seedlings were labelled, about 10 days after sowing and before the first leaf had expanded, by standing them with their washed roots in 0.1 to 0.8ml of undiluted tracer (containing 2 mci) according to the concentration of the tracer. When the seedlings had taken up most of the liquid, tap-water was added to maintain approximately 2 ml of liquid throughout the experiment. Seedlings were used in experiments 3 to 6 days after labelling (see p. 22).

Definition of probing used herein

Probing by aphids on labelled or diseased plants was as carefully defined as possible. For inclusion in experiments, aphids must have (a) started to probe during their first 15 sec. on the access-plant, (b) maintained contact between rostrum and leaf surface for the full duration of the probe, and (c) either rotated about the rostrum, or had developed a flexed rostrum, when disturbed before removal. Aphids which at any stage failed to maintain these conditions were rejected. Rejection rates

were often high, but this was accepted in order to maintain well defined treatments. Several aphids were allowed to start probing and usually only 1 to 3 were retained. Inoculation-probes on the test-plants were less rigourously defined. Aphids were placed on seedlings or leaf discs, observed to start probing and watched for 1 min. They were then left unobserved for the remainder of the inoculation probe. Aphids which walked from plants were rejected unless otherwise stated.

Assay for tracers

A Nuclear Chicago C115 gas-flow detector and scaler, or a Berthold sample changer fitted with either a GM tube or a Berthold gas-flow detector, were used for tracer assay. Background counting rates are specified in the text and for the three instruments were on average 4.8, 7.2 and 5.6 counts per minute respectively for operation on the β-plateau. Measurement of count-rates were either count-controlled (time taken to detect 300 pulses) or time -controlled (number of pulses detected in 25 min).

For assay of tracer, whole aphids were glued to small paper discs whereas parts of dismembered aphids were mounted in wax films on halfpennies used as planchettes. Other samples were mounted between thin polythene sheeting ('Glad-wrap') and a wax-polythene sheeting ('Parafilm'). The two layers were

sealed around the edge using an electrically heated ring. The mounted samples were assayed with the uncovered or polythene-covered sides towards the detector.

Presentation of data

Because the amount of tracer acquired by aphids can vary widely (from none, to very large amounts for feeding aphids), the variance of the mean is usually large and dependent on both the treatment and the mean. For this reason, comparisons are not often made between mean amounts of tracer; when means are compared treatment variances are used rather than an analysis of variance. More usually, treatments are compared using frequency distributions.

Logarithmic scales are used to reduce the effect of small numbers of aphids that carry large amounts of tracer. The use of logarithmic scales does not allow the subtraction of background, but was preferred because it allowed inclusion of all aphids in graphs. Text-figures therefore show the uncorrected count-rates. Background count-rates are specified either in the text or in the figures, sometimes in both.

The following scales are used:-

cp25m = number of disintegrations detected in 25 min; similarly $\log_{10}$cp25m.

cpm = the observed count-rate for the sample, without the subtraction of background, in counts per minute; similarly, $\log_{10}$ cpm.

ccpm = corrected count-rate as counts per minute, i.e. with background (BG) count-rate subtracted.

SAP UPTAKE AND EJECTION BY MYZUS PERSICAE

Introduction

A major problem in the study of non-persistent plant viruses is that there is no direct way known to measure the small amounts of virus carried or transmitted by aphids. It cannot be said that an aphid has acquired virus unless it can be shown to transmit the virus by probing a suitable test-plant In consequence, most ideas on the way that aphids transmit these viruses have been based on the conditions necessary for virus transmission, or that restrict it, rather than on direct observations on transmissible virus. Although sap transmission may not necessarily result in virus transmission, it presumably must occur when aphids transmit virus. Attempts were therefore made to observe sap transmission by aphids under condition which normally result in high frequencies of virus transmission.

Sap-uptake by M. persicae probing labelled plants

To determine whether sap-uptake occurred during probing, M. persicae which had been fasted for 2 hr were allowed 5-min probes on cucumber seedlings labelled with phosphorus-32 for different lengths of time. The amounts of tracer in the aphids were measured and are shown in Table 3 (p. 22).

Table 3. The effect of time between labelling and access-probes on the uptake of tracer by M. persicae probing cucumber. seedlings labelled with phosphorus-32.

Time after labelling	Tracer uptake (cp25m.)					
	Individual aphids					Mean
16 hr.	120	165	114	147	151	139.4
38 hr.	134	466	104	117	363	236.8
62 hr.	367	1181	1252	888	328	803.2
84 hr.	493	991	1574	904	739	940.2

Footnotes

Underscored figures are significantly larger than background taking P = 0.05.

The NC 115 detector was used and background = 120 cp25m.

The mean amount of tracer acquired increased with the time after labelling but the increase was much less during the third day than earlier. The numbers of aphids carrying tracer also increased during the first 3 days (3/5, 2/5 and 5/5 respectively). Although there was abundant tracer in the cotyledons probed on the first day, some aphids did not acquire any of it and others acquired only small amounts. The aphids would have probed only into the epidermis and underlying mesophyll cells, and the results may show that tracer movement into the epidermis is much slower than into the rest of the cotyledons.

The observed tracer uptake could have been from the surface of the plant and the aphids could have acquired it by direct contact with the labelled cotyledon. Watson & Nixon (1953) tested for this by allowing aphids to walk on wet filter paper, to which they transferred tracer. However, it is possible that aphids secreted tracer while walking on or attempting to probe the filter paper. Therefore I used other ways to study tracer uptake by probing aphids.

Pattern of tracer uptake

Individual M. persicae that had fasted for 2 hr were allowed to walk or probe for eight different lengths of time on a cucumber seedling labelled with phosphorus-32. The aphids

were randomly assigned to treatments in 5 replicates. The amounts of tracer in the aphids were measured and are shown in Table 4 (p. 25).

Aphids that had only walked on the labelled plant, even for 20 min, usually did not carry detectable amounts of tracer. Although a few aphids may have carried some tracer the amounts were small and a second assay of these often failed to detect the tracer. Only one aphid carried an amount significantly different to background at $P = 0.01$. However, many aphids that had probed the labelled plant carried large amounts of tracer.

Of particular interest were the aphids that probed for less than 11 min. All aphids that probed for 5 min carried tracer but those that probed for shorter or longer periods carried it less often, and usually in smaller amounts. This pattern was reproducible (Figs 1 to 4, p. 26) but the amounts carried, and the duration of probing that resulted in maximum uptake, often differed. Some aphids that probed for more than 11 min acquired very large amounts of the tracer (p. 25) and these may have started to feed on the phloem.

There are two likely explanations for the increase then decrease in the amount of tracer carried by aphids that probed for less than 11 min. They could have imbibed sap then ejected it during the longer probes. Alternatively, the tracer may have contaminated the stylets during probing but, after the

Table 4. Amounts of tracer carried by M. persicae which had probed or walked on labelled cucumber for different times.

Behaviour	Time in minutes	Log_{10} (cp25m.) per aphid					Mean (a)	S.d. of mean (a)
Probing	1	2.12	2.09	2.08	2.14	2.29	2.14	0.027
	3	2.02	2.10	2.14	2.04	2.42	2.14	0.065
	5	2.28	2.44	2.25	2.18	2.27	2.28	0.043
	7	2.19	2.12	2.07	2.08	2.25	2.14	0.034
	9	2.36	2.10	2.13	2.43	2.32	2.31	0.061
	11	2.26	2.13	2.08	2.14	(2.99)	2.16	0.066
	15	2.29	(4.57)	(4.41)	2.11	2.21	2.20	0.033
	20	2.30	(3.57)	(4.70)	2.56	(4.77)	2.43	0.058
Walking	1 to 9	2.07 (min.) to 2.17 (max)					2.10	
	11 to 20	2.09 (min) to 2.21 (max)					2.11	

Footnotes

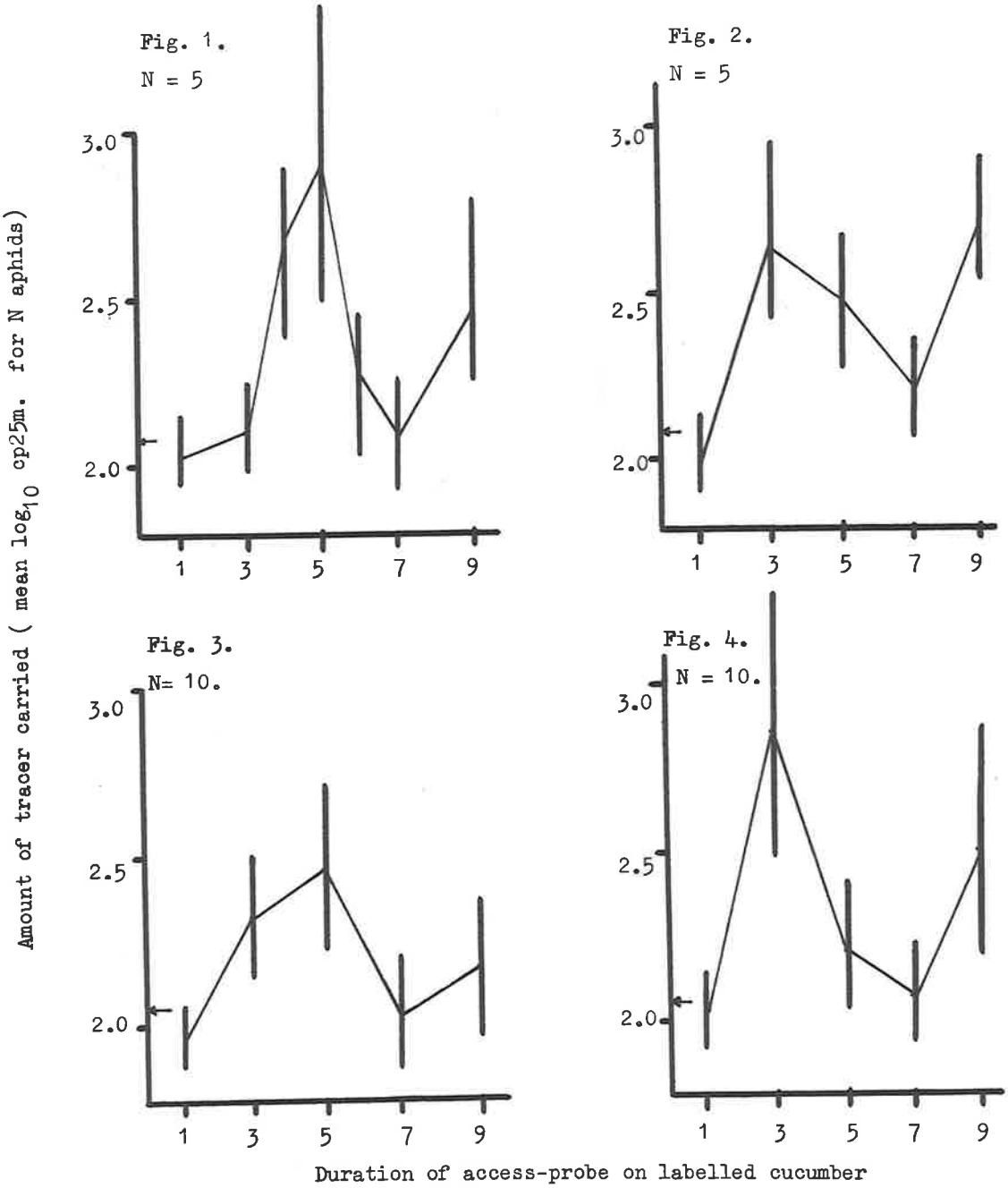
(a) These do not include the figures in paraethesis.

The large figures in parenthesis were considered to be exceptionally large and probably represented phloem feeding or attempts to feed.

Underscored figures represent aphids that carried tracer, taking P = 0.05

The NC 115 detector was used and background count-rate was 2.10

Figs 1 to 4. The effect of access-probe duration on the mean amount of phosphorus-32 acquired by M. persicae from labelled cucumber.



longer probes, contamination was reduced by the hardening salivary sheath.

Volume of sap imbibed

Consideration of the sap-volume carried by aphids that had probed for less than 10 min provides evidence that sap was imbibed and ejected during probing.

M. persicae that had fasted for 2 hr were allowed to probe a labelled plant for different times. The mean amounts of tracer carried are shown in Fig. 3 (p. 26). The labelled plant was mashed without added liquid and the pulp centrifuged at 10,000 g for 10 min to remove cell debris and the larger cell organelles. One µl of the supernatant fluid, after dilution to 10^{-6}, was assayed for tracer and yielded 6.3 ccpm. Most aphids carried this amount or more, and therefore probably more than 1000 μ^3 of sap. However this is a larger volume than can be accomodated by the food canal of the stylets.

The stylets slide over the cibarium which contains the anterior parts of the alimentary canal. Grooves in the two opposed maxillary stylets form extensions of the food and salivary ducts in the cibarium. Depending on the amount of extension of the stylets, the effective length of the food canal lies between approximately 60 and 200µ. During brief probes the stylets form a canal only about 60 to 80µ long. The

diameter of the food canal is approximately 0.5μ (Van Hoof,1958) and so the range of volumes it can accomodate are:-

minimum volume = $60 \times 0.25^2 \times 3.14 = 11.8\mu^3$

maximum volume = $200 \times 0.25^2 \times 3.14 = 39.3\mu^3$

Although the estimates of tracer concentration are liable to errors, caused in particular by probable uneven distribution of tracer in the leaf and in its cell components, the volume of sap carried by the aphids is far too large to be accomodated only in the food canals of stylets. Aphids did not acquire tracer while walking on labelled plants and therefore they probably imbibed sap, at least into the anterior part of the fore-gut, from which it was ejected during the longer probes.

The food duct of the cibarium leads to the much larger cibarial-pharangeal region which could easily accomodate the volumes imbibed. Therefore it is not necessary to postulate sap-ingestion for aphids that probed for less than 9 min. However, aphids in the first experiment of this chapter (p. 25) sometimes carried large amounts of tracer when they probed for more than 11 min. If the tracer concentration was of the same order as that of the last experiment, they may have carried as much as $300,000\mu^3$ of sap. These aphids must have ingested much of the sap they carried, but there is no evidence that they could or could not eject it. If aphids ingested tracer, ejected and secreted tracer would not easily be separated.

Distribution of tracer in dismembered aphids

Aphids were thought to imbibe sap into the fore-gut while probing plants. Additional evidence for this explanation of sap uptake was obtained by dismembering aphids and measuring the tracer in different parts of them.

After fasting for 2 hr, single M. persicae were allowed a 5 min access-probe on a cucumber seedling labelled with phosphorus-32. They were immediately mounted on their backs in warm wax on planchettes, and their parts removed using razor blade fragments. The rostrum and stylets were cut close to the head and mounted in a wax film on a planchette. The six legs were then removed and mounted together on a single planchette. The three samples (rostrum, legs and bodies) were assayed for tracer and the results are shown in Table 5 (p. 30). Eight aphids that had walked for 5 min on the labelled plant (but not probed) were also examined but tracer was not detected in any part.

The more tracer the aphids carried, the more often it was detected in bodies and rostra. Most aphids carried less than 40 ccpm and tracer was detected more often in bodies than in mouthparts. None of the aphids that carried less than this amount carried it on their legs. Because the tracer presumably entered the bodies via mouthparts, its detection might be expected at least as often in the mouthparts as in the

Table 5. Frequencies of tracer detection in parts of 48 dismembered M. persicae which had probed labelled cucumber for 5 minutes.

Class	Amount of tracer in aphid. (a)	Tracer detection in :-					
		Bodies		Mouthparts		Legs	
		+	-	+	-	+	-
1	2.11 to 2.40	16	2	3	15	0	18
2	2.41 to 2.70	10	1	4	7	0	11
3	2.71 to 3.00	4	0	2	2	0	4
4	3.00 or more	7	0	7	0	7	0
Totals		37	3	16	24	7	33

Footnotes.

(a) Amount of tracer expressed as $\log_{10}$ (cp25m.). Class 4 contains aphids that carried more than 40 cpm

The NC 115 detector was used and background = 2.01 ($\log_{10}$ cp25m) and the sample least significantly greater than BG is 2.11 (@ P= 0.05)

Eight aphids did not carry tracer with any part.

bodies. However, if the stylets were full of tracer they would only carry 1 ccpm or less (p. 27,28) and this is less than the smallest amount that could be detected with the counting methods used. Therefore, it is more likely that tracer in the rostrum is overestimated than under estimated.

Overestimation of the tracer in rostra and stylets could have arisen in three ways:

(a) by spillage of tracer from bodies during dismemberment, or involuntary sap-ejection during death.

(b) by accidental inclusion of the anterior part of the cibarium with the rostra and stylets.

(c) by ingestion, assimilation and transport of tracer in the haemolymph.

The first of these possibilities could not be tested. The anterior part of the cibarium was difficult to detect in the amputated rostra, but was observed for five of the aphids that carried tracer with mouthparts, and two that did not carry large total amounts. Aphids that had not probed did not carry tracer and most that probed did not carry tracer with their legs. Those that did carry tracer with their legs carried it with all parts and carried more than about 40 ccpm. These aphids had probably ingested some of the tracer which was assimilated and had migrated in the haemolymph to most of their parts.

Although there is doubt as to how tracer became associated

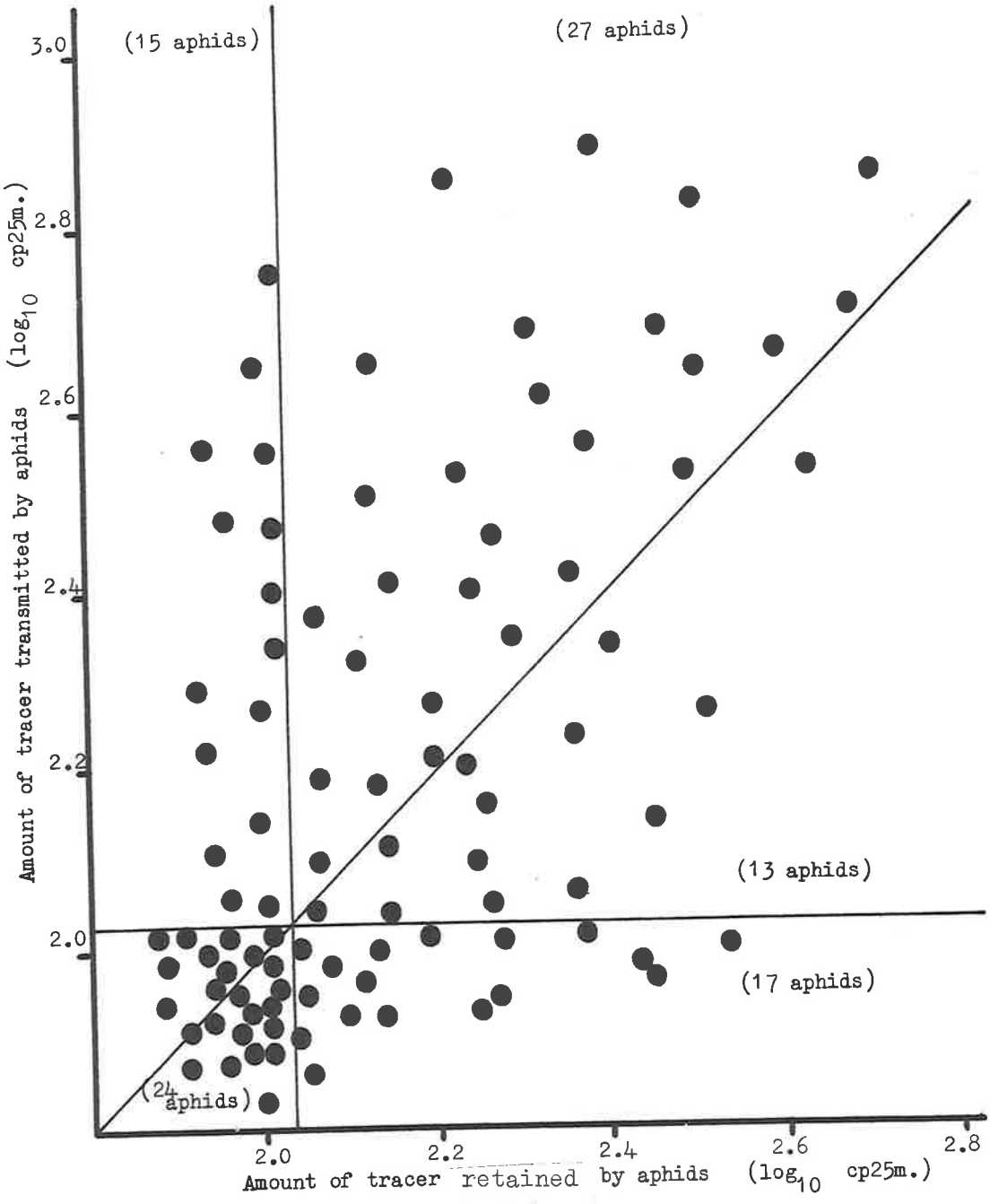
with mouthparts, its overestimation seems likely and most aphids imbibed sap which they carried in their bodies, presumably in the cibarium. Some aphids had ingested tracer in large amounts, even though they were unlikely to have fed on the phloem during the 5-min. probes they made.

Sap transfer to leaf discs.

The experiments described have shown that aphids imbibe sap while probing, and the pattern of uptake showed that they can eject some of it during single probes (p. 25). To determine whether they could eject the imbibed sap during subsequent probes, fasted aphids were allowed to probe labelled cucumber cotyledons for 5-min. then to probe 12-mm discs, cut from unlabelled cucumber cotyledons, for 3 min. the discs were mounted on pins inserted into water flooded agar, but were several mm above the water. Separate discs were used for different aphids. When aphids walked from the discs they were trapped in the water; these aphids and the corresponding discs were rejected. Only the first minute of the inoculation probes, but the whole of the access probes, were critically observed (p. 17). Aphids and discs were assayed for tracer and the results are shown in Fig. 5 (p. 33).

Most aphids transferred large proportions of the tracer they carried. Of the 96 aphids in the experiment, 15 transferred

Fig. 5. Tracer acquisition and transmission by M. persicae.



all the tracer they carried, 42 transferred more than 50%, 30 transferred less than 50% and 17 transferred none. Tracer was not detected in 24 aphids, nor in the discs they probed.

The fact that many aphids had, in a single inoculation probe, transferred a large proportion of the tracer they carried, makes it unlikely that they transferred tracer with saliva. Secretion of tracer with saliva would require its prior assimilation and most of itwould be expected to remain in phosphate pools within the aphids. Lamb,Ehrhardt and Moericke (1960) found that aphids which had fed for long periods on plants labelled with Rb^{86}, and would have ingested most of the tracer they carried, were able to secrete only 1 to 2 % of the tracer during long inoculation probes. In their experiments, Lamb et al. (1960) chose experimental conditions which ensured that saliva was heavily labelled. In the present study, the aim was to study tracer transmission other than by its secretion with saliva and experiments were designed to keep tracer assimilation to a minimum.

To test whether retained tracer was transferable, aphids which had probed labelled plants for 5-min were each allowed a sequence of 3-min inoculation probes on 5 cucumber cotyledon-discs. The detection of tracer in the aphids and discs is recorded in Table 6 (p. 35). Inoculation probes did not necessarily lead to the transmission of tracer to the discs. However, failure to

Table 6. Phosphorus -32 transmission by M. persicae during successive 3-min. inoculation probes.

Aphid	Detection of tracer (+) in 5 successive probes (1 to 5)					Detection of tracer in aphid.
1	+	-	-	+	-	+
2	+	-	+	-	+	+
3	-	-	-	+	+	-
4	-	-	+	-	-	-
5	-	+	-	-	-	-
6	+	-	-	-	-	-
7	+	-	-	-	-	-
8	-	+	-	-	-	-
9	-	-	+	+	-	+
10	-	-	-	+	-	+
11	-	+	-	+	-	+
12	-	-	+	-	-	+
13	+	-	-	+	-	-
14	-	+	-	-	-	+
Totals	5	4	4	6	2	7

(+) Count rate exceeded 134 cp25m.

(-) Count rate less than or equal to 134 cp25m.

134 cp25m. is the least significant count rate (@ P = 0.05) using the NC 115 detector (BG = 125 cp25m.).

transmit tracer did not indicate a permanent inability to do so. Twelve of the fourteen aphids that acquired tracer, transmitted tracer after failing to transmit it on a previous opportunity. One aphid failed to transmit on three successive occasions then transmitted all that it carried.

The results confirmed that the aphids could transmit large proportions of the tracer they acquired, but showed that they could retain transferable tracer for long periods while probing. This result is compatible with the earlier ones which showed that aphids probably carried transmissible tracer within the cibarium.

Comparison of tracer in the rostrum with tracer transferred to leaf discs.

The sap transmission and the aphid dismemberment treatments described above were combined in a single experiment. This was done to confirm the results of the previous experiments and to allow direct comparisons between the two treatments.

Aphids that had fasted for 2 hr before use were randomly assigned in the ratio 2:1 to the two treatments. The methods used were those described on pages 29 and 32. Ninety-six aphids were assigned to the sap transmission treatment and 48 to the dismemberment treatment. The amounts of tracer acquired by the aphids of the two treatments were similar (Table 7, p.37) and the difference between the proportions of aphids transferring

Table 7. Comparison of tracer uptake in transfer and dismemberment treatments.

Amount of tracer carried	Number of aphids in :-	
	Transfer series (a)	Dismemberment series (b)
2.11 to 2.30	17	5
2.31 to 2.50	30	20
2.51 to 2.70	27	14
2.71 to 3.00	13	4
Totals	87	43

Chi-square (3 df) = 2.87, P = 0.3

Footnotes.

(a) Six aphids did not carry tracer; 3 aphids carried more than 40 cpm

(b) Two aphids did not carry tracer; 3 aphids carried more than 40 cpm

Table 8 Comparison of frequencies of tracer transfer and tracer detection on mouthparts.

Treatment	Tracer detection		Totals
	+	-	
Leaf discs of transfer series	59	28	87
Mouthparts of the dismemberred series	11	32	43
Totals	70	60	130

Chi-square (1 df) = 20.65 ; P <<< 0.01

tracer and carrying it in their rostra and mouthparts was confirmed (Table 8, p. 38). In this experiment, the aphids that failed to acquire tracer and those that carried more than 40 ccpm were not included in the analyses. However, their numbers were small (Table 7, p. 37) and would not affect the conclusions that:

(a) aphids imbibe sap into the food canal and at least the anterior part of the fore-gut;

(b) aphids can eject sap carried in these parts.

SAP AND VIRUS TRANSMISSION BY APHIDS

Introduction

The transmission of non-persistent virus presumably requires transmission of plant sap. However, the mechanism of sap-uptake and ejection described in the previous chapter is not necessarily the same as that of virus transmission. It is possible that aphids were ejecting sap at sites in plant tissues, or on the plant surface, where infection could not occur. It is also possible that virus is inactivated or inhibited by secretions carried in the cibarium and ejected with the virus. Therefore, studies were made to determine whether virus and tracer transmission by aphids depended on the same factors, and to the same extent.

Attempts to transmit tracer and virus from the same plant

Several cucumber seedlings at the cotyledon stage were manually inoculated with QCMV and 10 days later developed symptoms on the first leaf. One of the infected plants was labelled with 2 mci phosphorus-32 for 3 days. Groups of about 20 *M. persicae* which had fasted for 2 hr. then allowed 5-min access-probes on the first leaf of either the labelled or one of the unlabelled plants. Aphids were transferred to small *N. glutinosa* plants and allowed to probe or feed overnight. The test seedlings probed by aphids from the labelled plant were mounted on planchettes without the aphid (p. 18), assayed for tracer and planted for symptom assessment. Those probed by aphids from the unlabelled plant were grown without prior assay. The treatments were applied in random order to the successive groups until 40 aphids had been assigned to each treatment.

Twenty-one of the aphids from the unlabelled plant, but none from the labelled plant, transmitted the virus. The difference between the treatments could have been caused by the interactions of either the tracer or the storage conditions during assay, with either the infectivity of the virus or the infection of the test-plants. However, sap-inoculation from the labelled plant to cowpea produced no local lesions on 6 half-leaves whereas sap-inoculation from the unlabelled plant produced 130 lesions per half-leaf. The experiment was repeated twice, using phosphorus-32

or sulphur-35. Similar results were obtained; it was not possible to transmit virus from the labelled plants using either aphids or sap inoculation. The tracer presumably inactivated the virus in the labelled plants. Schlegel, Gold & Rawlins (1953) reported similar inactivation of TMV in tobacco plants labelled with smaller amounts of phosphorus-32 than were used in my experiments.

The study of tracer and virus transmission by single aphids would be particularly useful to establish a dose-response relationship between sap and virus transmission. Many attempts were made to improve the counting efficiency of the detectors, and to reduce the amount of tracer used in the access-plants. However, a large reduction in the radiation damage to the virus could not be obtained for several reasons:

(1) Aphids often acquired only small amounts of tracer when probing plants labelled with 2 mci phosphorus-32. A large decrease in the amount of tracer would require large improvements in the efficiency of tracer assay.

(2) A large increase in the ratio of efficiency squared to background could not be obtained.

(3) The counting period for a sample was 30 to 60 min. The decrease of tracer concentration in the access-plant required unacceptably long assay times which also increased the numbers of seedlings that died.

(4) Reduction of the time between labelling and access-probes

decreased the amount of tracer available to the aphids (p.22) and had no effect on virus survival over the range that it could be used.

This experimental approach was therefore discontinued. Instead, pre-access fasting and access-probe durations were studied for their effects on the transmission of virus and tracer from separate plants, using several aphid species.

Behaviour of different aphid species

Six aphid species were used at different times for studies on their behaviour during probing, as measured by tracer uptake and virus transmission. A good correlation between these parameters is not to be expected. However, the measurement of behaviour during probing by measuring sap-uptake is sufficient for deciding whether different aphid species are affected by access-probe duration in different ways. Furthermore, reliable measurement of sap-uptake requires fewer aphids than does reliable measurement of sap-transmission to leaf-discs or seedlings. For these reasons, the effect of access-probe duration on sap-uptake and virus transmission was studied for several aphid species and detailed studies of sap and virus transmission reserved for later experiments.

The six aphid species were studied in the same way. Groups of 20 aphids that had fasted for 2 hr were allowed to probe for 1, 3,

5, 7 or 9 min on plants labelled with phosphorus-32 or unlabelled plants infected with QCMV or PVY. The groups of aphids were randomly assigned to the 10 treatments and all aphids of a group received the same treatment. Only 3 to 6 of the aphids of each group probed according to the standard requirement (p. 17) and the experiment was repeated until 10 aphids had probed the labelled plant and 20 had probed the infected plant. Aphids that had probed the labelled plants were assayed for tracer and those that had probed the unlabelled plants were allowed a 3 min inoculation-probe on test-seedlings susceptible to the virus used.

Myzus persicae (Fig. 6, p. 44) and Hyperomyzus lactucae (Fig. 7, p. 45) were efficient vectors of QCMV and also readily imbibed tracer. Maximum transmission of virus, and maximum tracer uptake, occurred after access-probes lasting 3 to 5 min. In both experiments the effect of access-probe duration was similar to that described in earlier experiments on tracer uptake (Fig. 1 to 4, p.26). However, the decrease in the mean amount of tracer carried by M. persicae after the longer access-probes was not significant, largely because of a marked increase in the standard deviation of the mean. For both species, the decrease in frequency of virus transmission is slightly more marked than the fall in numbers of aphids carrying tracer. Also, in both experiments, the frequency of tracer detection is larger than the frequency of virus transmission and this suggested that the extra labour involved in studying sap and virus transmission

Fig. 6. Tracer uptake and virus transmission by M. persicae.

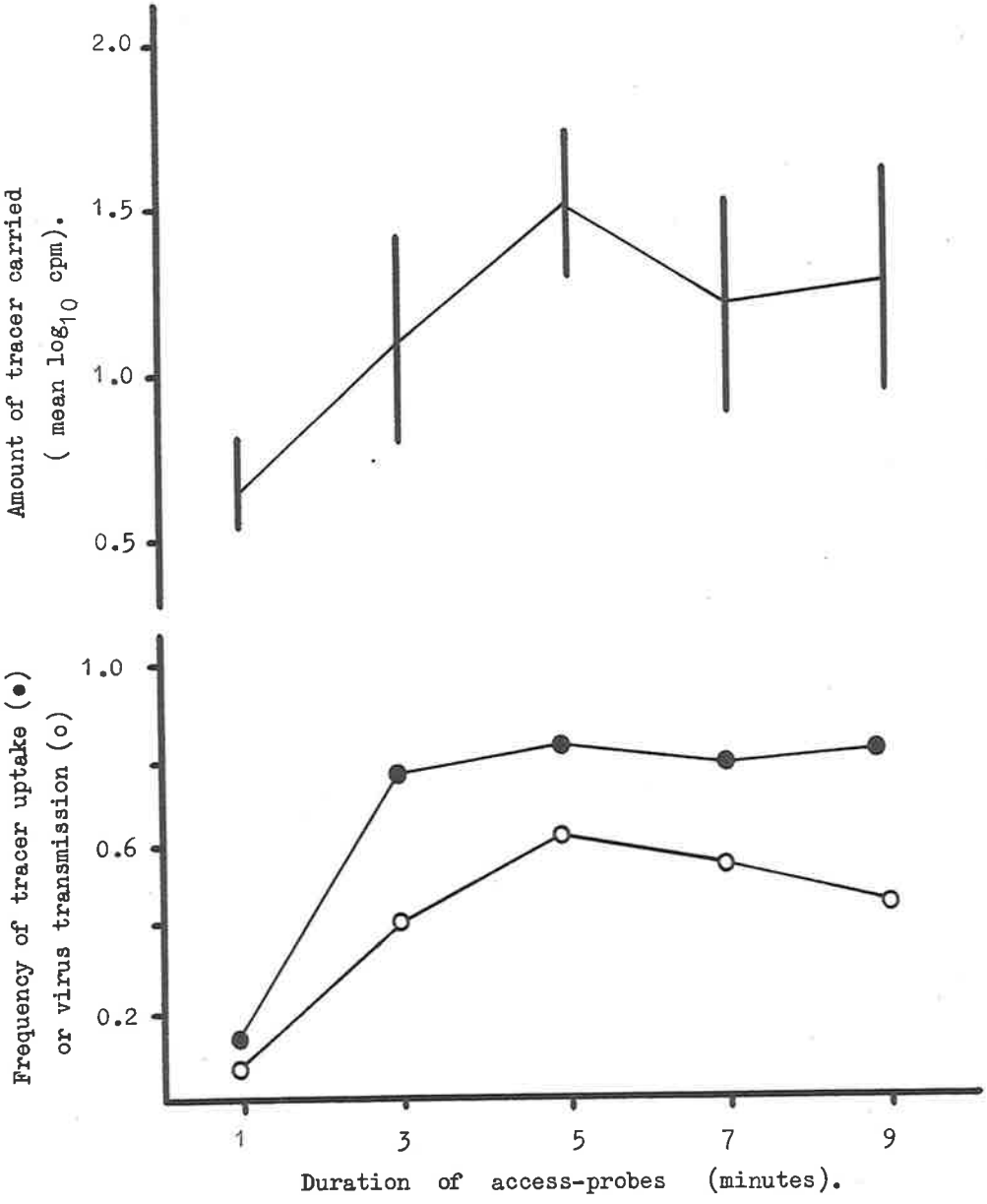
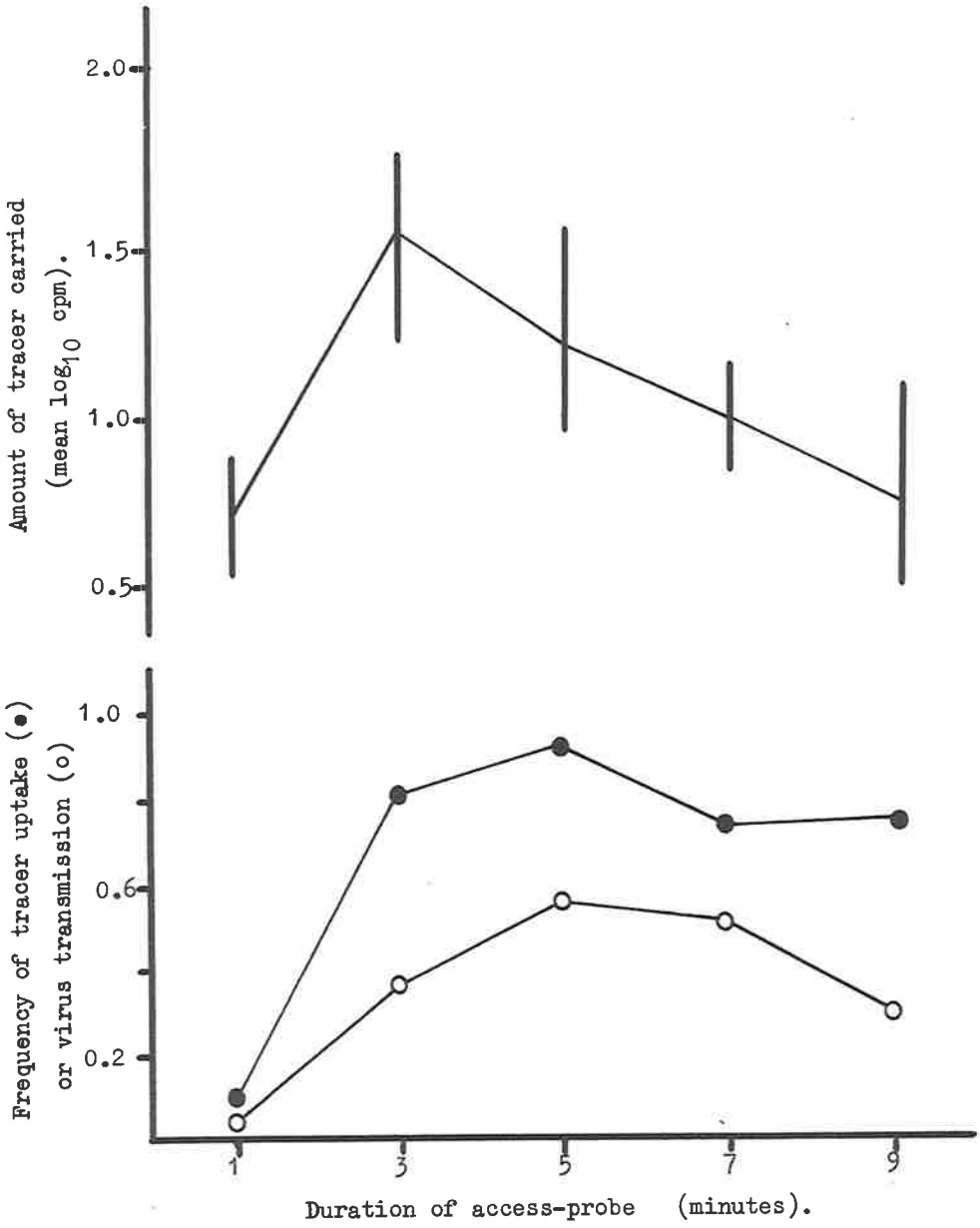


Fig. 7. Tracer uptake and virus transmission by H. lactucae.



would probably be worthwhile. This was done in experiments reported in the next section. The transmission of PVY was not studied using these aphids.

In contrast to M. persicae and H. lactucae, Acyrthrosiphon solani and Macrosiphon euphorbiae were poor vectors of both PVY and QCMV. In separate experiments, 8/140 A. solani transmitted QCMV and 5/140 transmitted PVY. Similarly, 3/140 M. euphorbiae transmitted QCMV and 5/140 transmitted PVY. These aphids were of particular interest as they were also inefficient at sap- uptake. Only 16% of M. euphorbiae and 19% of A. solani carried more than 6.6 cpm after probing the labelled plants, although some carried large amounts of tracer. Under the assay conditions used, 5% of the background counts are expected to exceed 6,6 cpm.

A simple relationship between sap-uptake or virus transmission and the duration of access-probes could not be demonstrated for Aphis craccivora (Fig. 8, p.47; Table 9, p. 49). However, the aphid was efficient at both sap-uptake and virus transmission. The standard deviations of the mean amounts of tracer carried were often large and individuals of the species probably vary greatly in their behaviour. In an experiment using Aphis gossypii (Fig. 9, p. 48; Table 9, p.49), the aphids were similarly found to be variable, but efficient at both sap-uptake and virus transmission. For this species, the large value of chi-square suggests that the duration of access-probe affected both sap-uptake and virus transmisiion.

Fig. 8. Tracer uptake and virus transmission by A. craccivora.

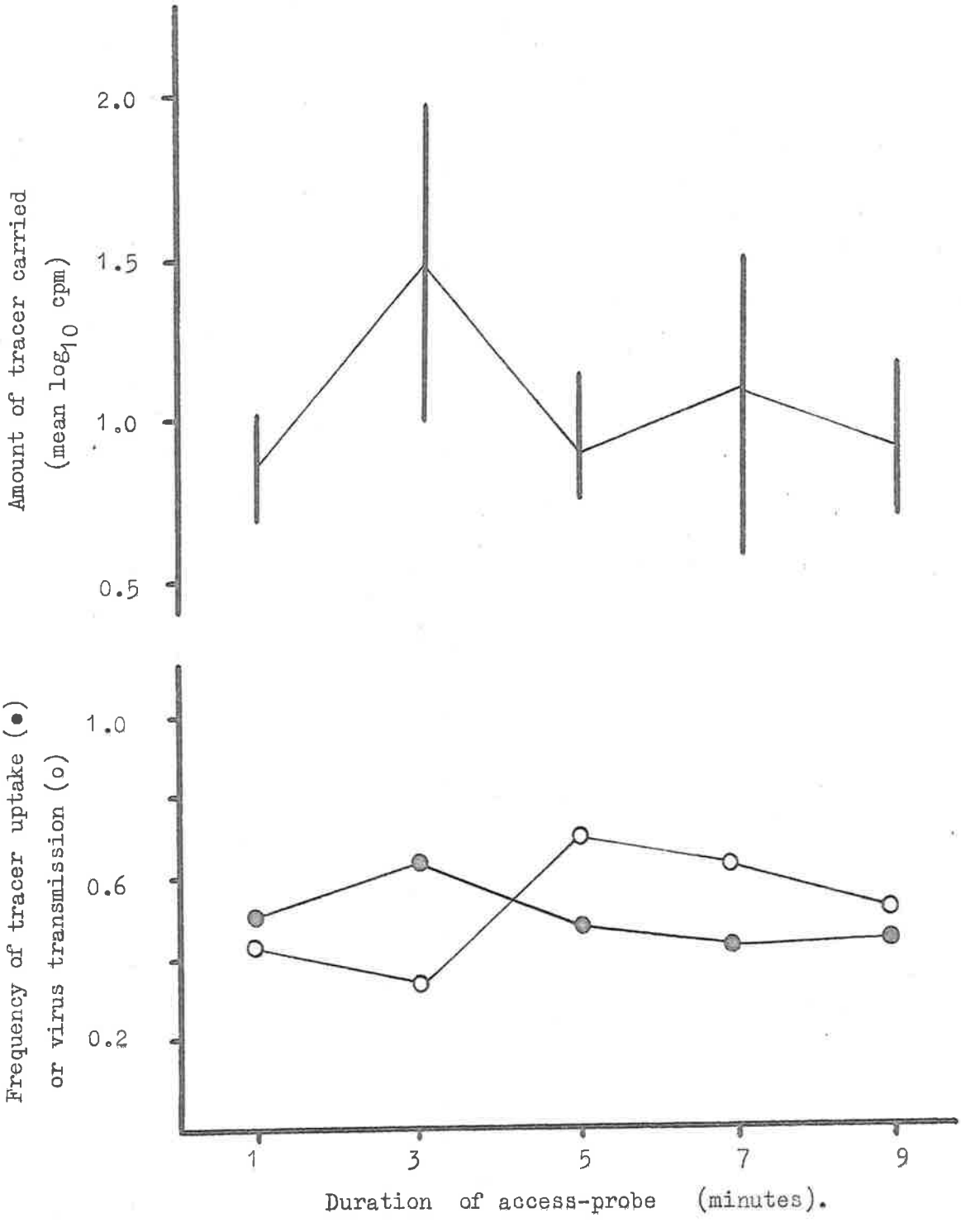


Fig. 9. Tracer uptake and virus transmission by A. gossypii.

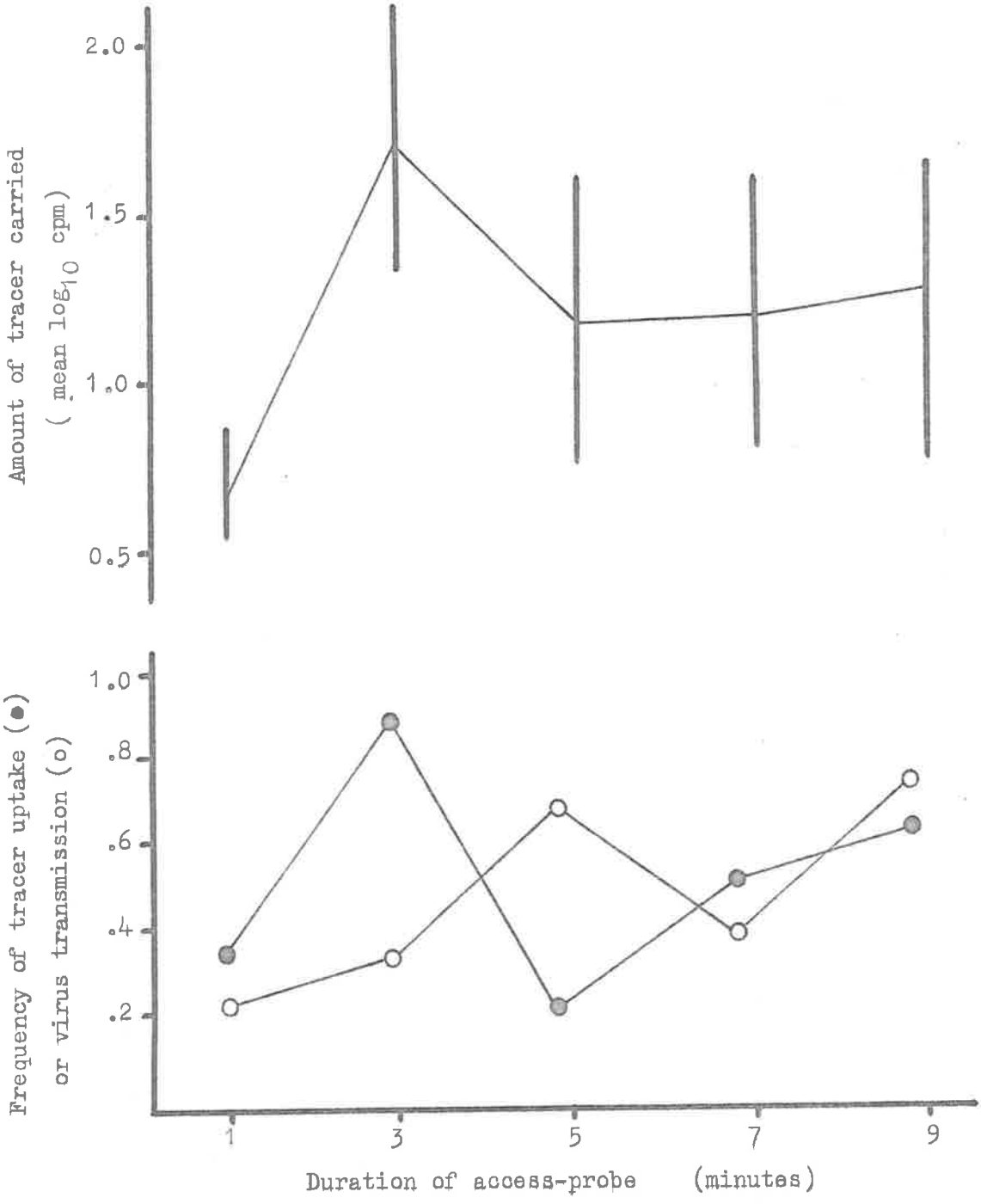


Table 9. The effect of probe duration on the frequency of sap-uptake and virus transmission for four aphid species.

Species	Text fig.	Chi-square and P for :-	
		Sap-uptake	Virus transmission
M. persicae	7	15.8	17.7
		.01 > P > .001	.01 > P > .001
H. lactucae	8	16.6	13.6
		.01 > P > .001	.01 > P > .001
A. craccivora	9	2.09	2.99
		.80 > P > .70	.70 > P > .60
A. gossypii	10	10.51	17.0
		.05 > P > .02	.01 > P > .001

Footnote

Although the large value for chi-square for A. gossypii suggests that access-probe duration affected both tracer and virus transmission, this is not certain for reasons discussed on page 46.

However, since there is not a simple trend in the relationships, and because variances for tracer uptake are consistently larger than for the other aphid species, it is not certain that the effects could be satisfactorily described without using very large numbers of aphids. Because these two species are not grouped by access-probe duration into different but homogeneous classess of aphids they were not used in further experiments.

Sap-volume transmitted during virus transmission

Of the six aphid species studied, only M. persicae and H. lactucae were suitable for a detailed study of the relationship between sap and virus transmission. They consistently carried tracer more often than they transmitted virus, and different access-probe durations provided groups of aphids that differed in the amounts of tracer they carried and in their efficiencies of virus transmission. By using these properties, an estimate was obtained of the sap-volume they transmitted while transmitting virus.

(a) Method of estimation

To establish a dose-response relationship between sap and virus transmission by probit analysis would require the simultaneous transmission of virus and tracer by individual aphids. This was not possible because the tracer inactivated the virus in the labelled plant (p. 39). The mean amount of

tracer transmitted by aphids from a labelled plant could be compared under different conditions with the frequency of virus transmission from unlabelled plants. However, this was considered to be unsatisfactory. The mean amount of tracer transmitted is a poor estimate of, for example, the effect of access-probe duration on aphids (p. 19). The majority of aphids acquire only small amounts of tracer, but the mean is markedly affected by the relatively few that imbibe large amounts. Its use would therefore overestimate the amount of sap transmitted by most of the aphids. Transformations do not satisfactorily solve this problem.

Instead, an amount of tracer was chosen for each experiment such that the number of aphids that transmitted it (or more) from the labelled plant equalled the number that transmitted virus, irrespective of the treatment to which the aphids were assigned. This amount of tracer (CT) and the sap-volume it represented, were thus dependent on the concentrations of both the tracer in the labelled plant and the virus in the unlabelled plant. The number of aphids that transmitted CT or more could be compared for each treatment of the experiment, with the number that transmitted virus. This provides a test of whether sap and virus transmission depend to the same extent on the various treatments, as they must if they represent the same phenomenon.

Four experiments were carried out, two with M. persicae

and two with H. lactucae. In the first experiment with each species, only the duration of the access-probe was varied. In the other experiments, both pre-access fast and access-probe durations were varied. A fifth experiment, using M. euphorbiae resulted in only a few aphids transmitting virus or tracer.

(b) Experimental details

For each experiment, a cucumber seedling infected with QCMV was labelled with phosphorus-32, 12 days after it had been inoculated. A similar seedling was retained unlabelled. Four days later, groups of about 20 aphids were collected, stored if necessary, then allowed various access probes on either the labelled or unlabelled plant. Three-minute inoculation-probes were allowed on either cucumber seedlings or cotyledon-discs, for virus or sap transmission respectively. Cotyledon-discs were assayed for tracer in the usual way (p. 18). Only 4 to 7 of the aphids in each group satisfied the requirements for access-probes (p. 17). Treatments were applied to successive groups in random order. When aphids were fasted for 2 hr. only, the access-probes lasted for 1, 3, 4, 5, 6, 7, or 9 min and two replicates of each of the fourteen treatments were carried out on successive days until 40 aphids had been assigned to each treatment. When pre-access fast was also varied, one complete replicate was carried out on successive days until 30 aphids had been assigned to each treatment.

In these experiments, pre-access fasts lasted for 0, 1, 2, or 3hr and the access-probes lasted for 1, 3, 5, 7, or 9 min. Experiments required 6 to 8 days to complete and tracer assays were made usually within 24hr of the access-probes. However, with the larger experiments, there was some build-up of samples for assay; but the delays before assay were always less than 48 hr. During this time tracer decay was considered trivial compared with the variation between aphids. Also, the transmission of tracer did not show a consistent fall for successive days, as would be expected for tracer decay in the labelled plant. It is likely that tracer redistribution in the labelled plant offset the effect of tracer decay. Because a correction for decay would have necessitated an equal but opposite correction for tracer redistribution, no correction was applied.

(c) Experiment I

In this experiment, M. persicae was used and only the duration of access-probes was varied.

One hundred and forty-seven aphids transmitted QCMV. The cumulative distribution curve for tracer transmission by the 280 aphids which probed the labelled plant is shown in Fig. 10 (p.54). The estimate of CT was 4.2 ccpm (1.0 = $\log_{10}$ 10 cpm; background = 5.8 cpm), 147 aphids transmitting this amount or more. The concentration of tracer in the labelled plant (which was measured

Fig. 10 Cumulative distribution curve for tracer transmission by M. persicae.

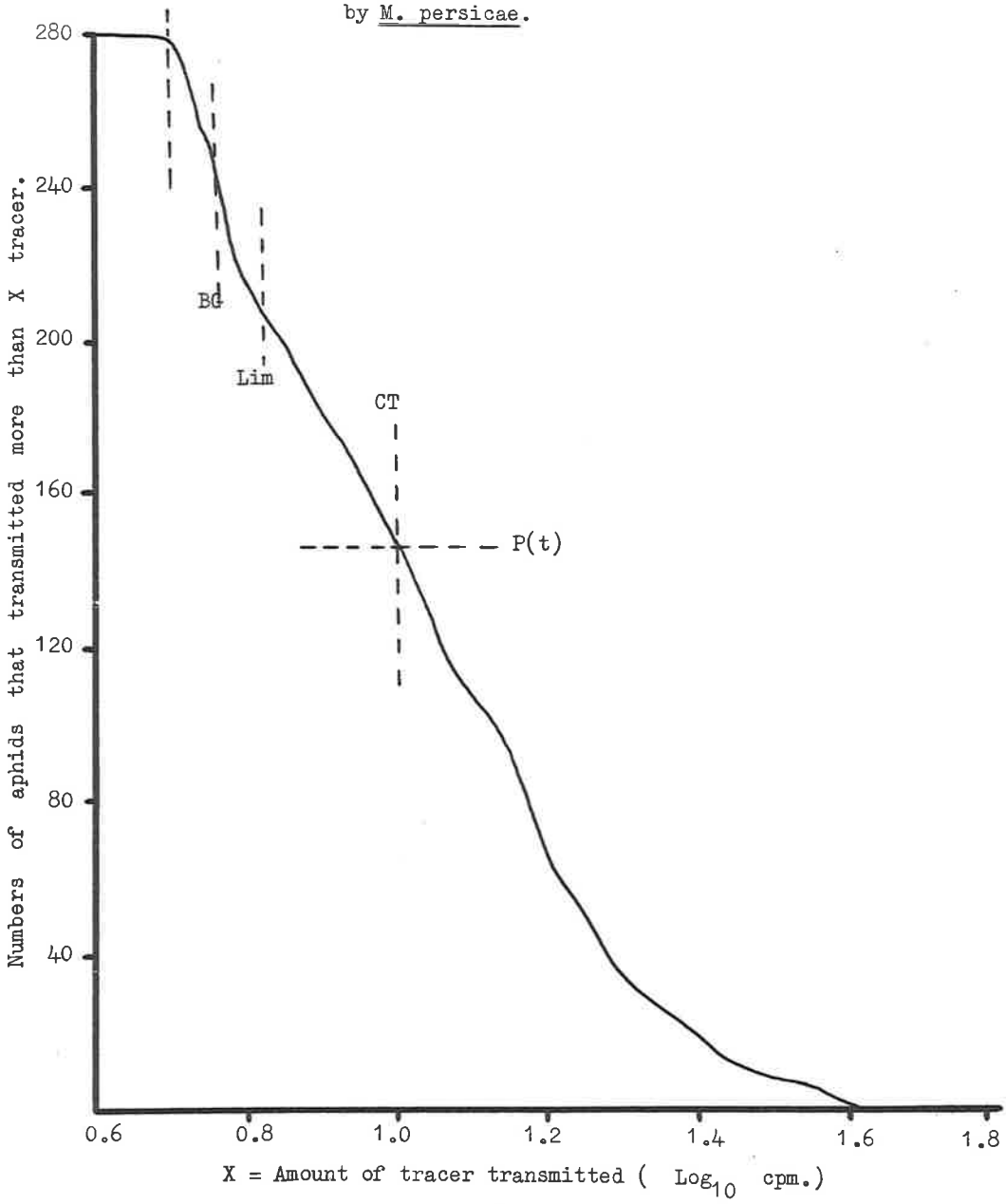


Table 10. Comparison between the numbers of M. persicae that transmitted more than 4.2 ccpm (= 570 μ^3) and the numbers that transmitted virus in experiment I.

Duration of access-probe (min)	Aphids per treatment.	Numbers that transmitted :- QCMV	4.2 ccpm or more.
1	40	9	8
3	40	21	20
4	40	26	22
5	40	28	33
6	40	26	27
7	40	19	20
9	40	18	17
Totals	280	147	147

Chi-square = 2.10 (6 df); P > 0.9

in the manner described on p. 27, and with the same limitations) was 7.4 ccpm per 1000 μ^3 and the sap-volume which represented CT was approximately 570 μ^3. The numbers of aphids that transmitted this amount or more are compared in Table 10 (p. 55), for each access-probe duration, with the numbers that transmitted virus. The estimate of CT was found to be satisfactory for all the access-probes; the largest discrepancy, for access probes lasting 5 min, is not statistically significant.

The alternative approach, based on the mean amount of tracer transmitted, did not give a linear relationship between sap and virus transmission whereas the method adopted did (Fig. 11 & 12; p. 57), presumably for the reasons discussed on p. 50.

Aphids transmitted up to 10 times the volume estimated as CT. This allows aphids to transmit on several occasions, or to transmit less than that amount on some occasions but retain sufficient sap for subsequent transmissions.

(d) Experiment II

H. lactucae was used and only the access-probe duration was varied. The derivation of CT is shown in Fig 13 (p. 58), and the comparison between numbers of aphids transmitting more than this amount, with the numbers transmitting virus, is shown in Table 11 (p.59) . The correlation between sap and virus transmission was not as good as for M. persicae (p. 55) in the preceeding

Tracer and virus transmission by M. persicae.

Fig. 11.

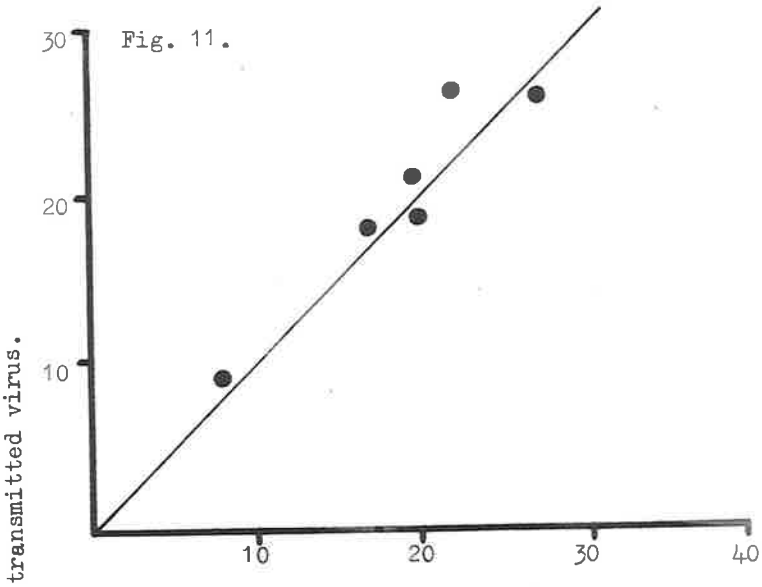


Fig. 12.

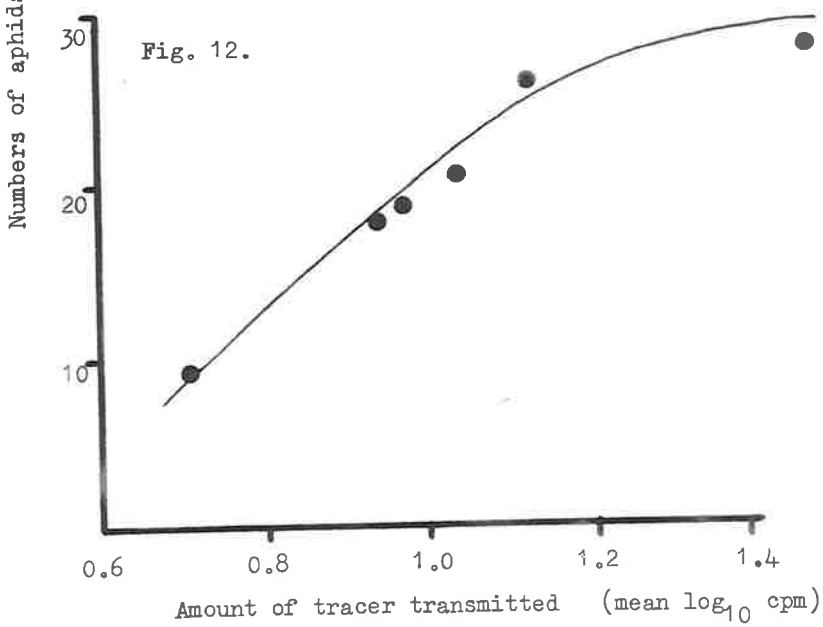


Fig. 13. Cumulative distribution curve for tracer transmission by H. lactucae.

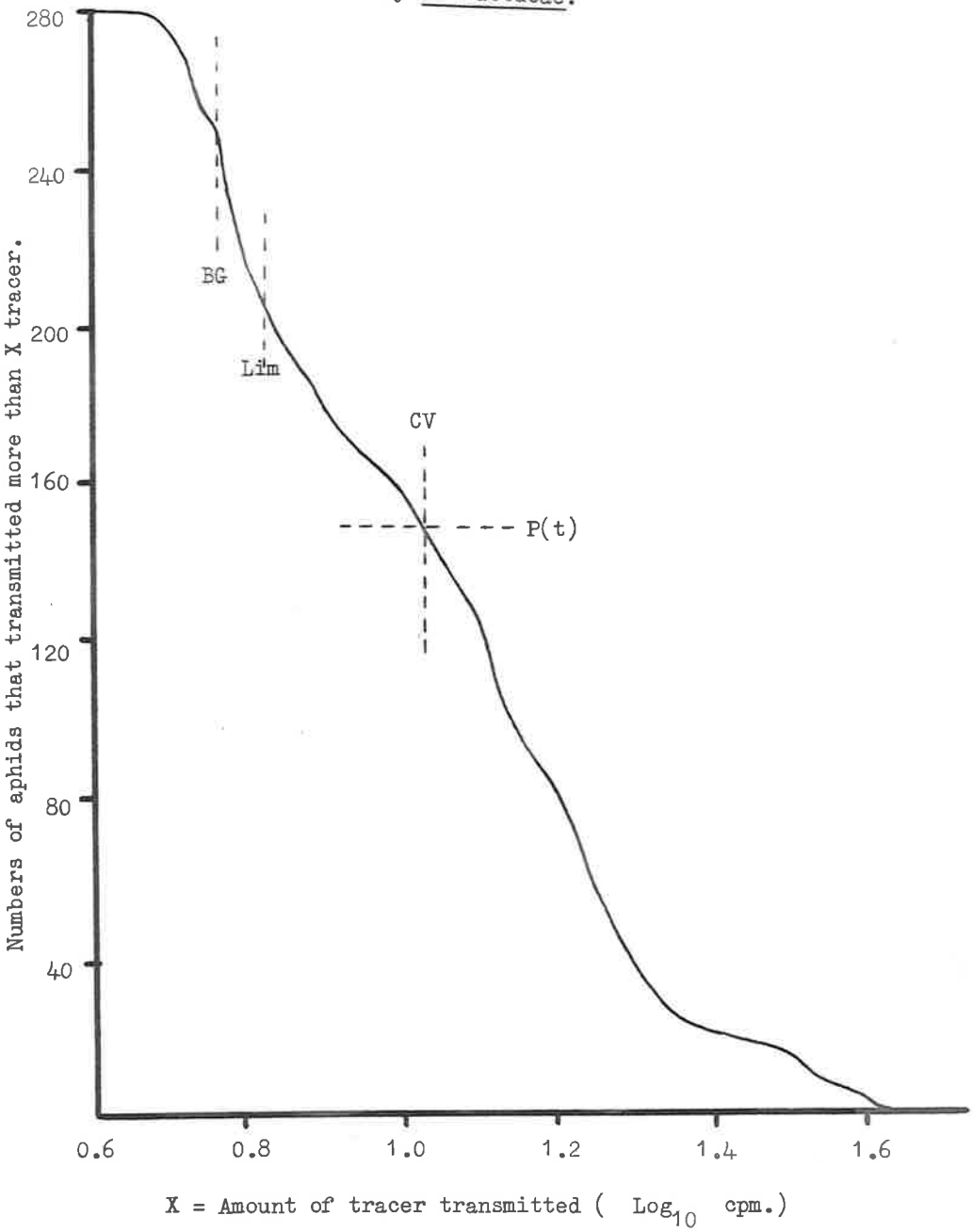


Table 11. Comparison between the numbers of H. lactucae that transmitted more than 4.0 ccpm (= 480 μ^3) and the numbers that transmitted virus in experiment II.

Duration of access-probe	Aphids per treatment	Numbers that transmitted :-	
		QCMV	4.0 ccpm or more
1	40	13	4
3	40	22	25
4	40	26	29
5	40	28	25
6	40	21	27
7	40	16	14
9	40	16	18
Totals	280	142	142

Chi-square for comparison between columns 3 and 4

= 4.90 (6 df); P> 0.5

experiment. However, this is largely because of the small numbers of aphids transmitting tracer after 1-min probes compared with those transmitting virus. The difference is not sufficiently large to cause a large value for chi-square for the comparison of the 7 treatments of Table 11 (p. 59). It is likely that the results are no more heterogeneous than might be expected for several comparisons between pairs of aphid populations. For this experiment, CT was 480 μ^3, not very different from that in the preceeding experiment.

(e) Experiment III

M. persicae was used and both pre-access fast and access-probe durations were varied. The value for CT was estimated from the number of aphids (223) that transmitted virus and the cumulative distribution curve and was found to be 4.19 ccpm. This corresponded to 640 μ^3 of sap as estimated in the usual way. The transmission of virus and tracer (Table 12, p.61) correlated well, and only one of the individual comparisons shows a large enough proportionate difference to attain significance. This is for unfasted aphids that made 1-min probes and the figures are too small for reliable estimation of chi-square.

Table 12. Comparison between the numbers of M. persicae that transmitted more than 4.19 ccpm (= $640\mu^3$) and the numbers that transmitted virus in experiment III

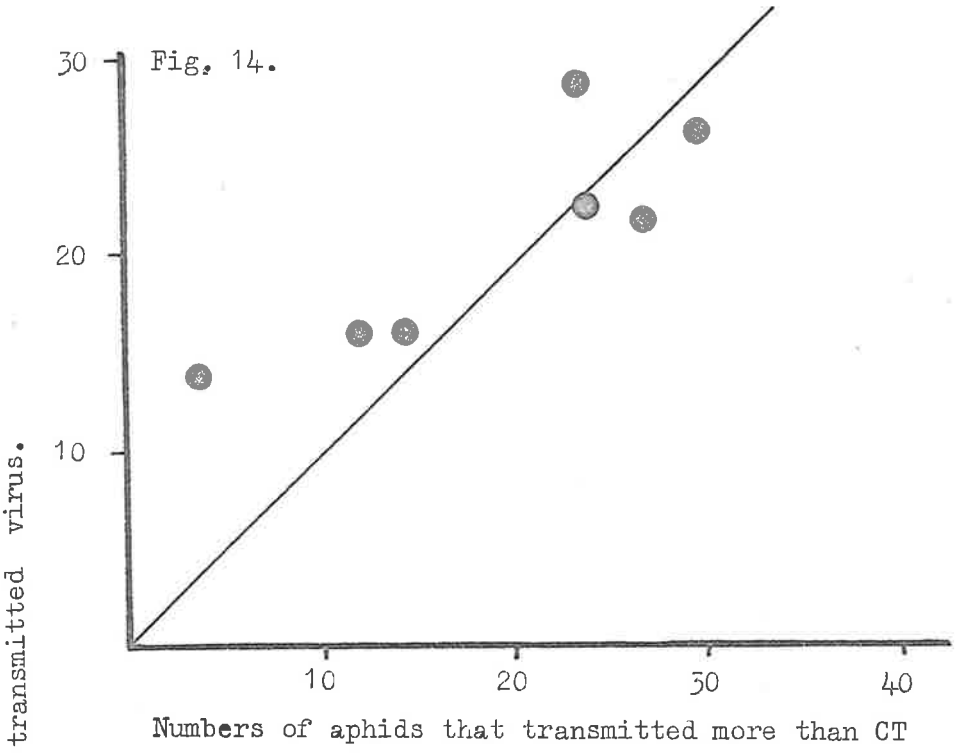
Duration of access-probe (min)	Duration of pre-access fast (hr)				Totals
	0 (a)	1	2	3	
1	5 : 1	12 : 10	11 : 15	9 : 10	37 : 36
3	7 : 9	18 : 14	16 : 14	17 : 12	58 : 49
5	6 : 8	13 : 16	21 : 18	16 : 14	56 : 56
7	8 : 6	10 : 13	14 : 17	11 : 9	43 : 45
9	7 : 8	11 : 8	12 : 14	9 : 7	39 : 37
Totals	33 : 32	64 : 61	64 : 78	62 : 52	223 : 223

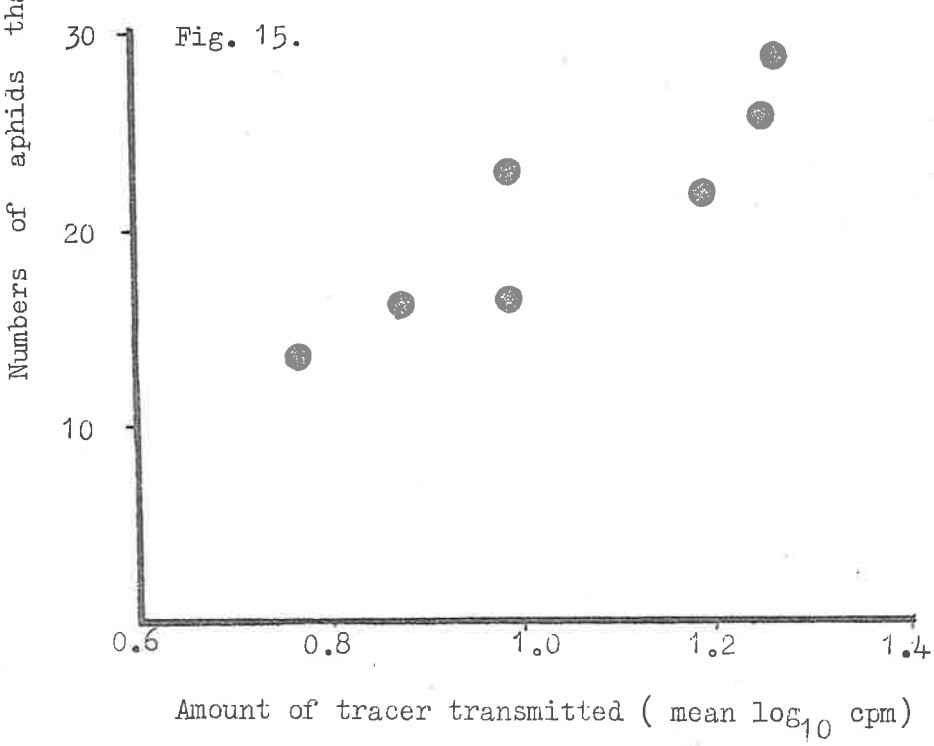
Footnote

(a) The results are presented as the ratio :-

transmission of virus : transmission of tracer

Fig. 14 & 15. Tracer and virus transmission by H. lactucae.





(f) Experiment IV

H. lactucae was used and both pre-access fast and access-probe durations were varied. Two hundred-and-four aphids transmitted virus and CT was estimated as 7.25 ccpm (= 841 μ^3). Aphids transmitted more tracer than in previous experiments. This was partly because the tracer concentration in the plant was higher than usual, and partly because the aphids imbibed larger volumes. The reasons for these differences are not known.

The correlation between sap and virus transmission was good (Table 13, p.64). The results showed a slightly larger range of differences for the individual comparisons but this does not lead to heterogeneity in the general comparison between sap and virus transmission. In making multiple comparisons, using each of the 20 treatments for separate tests, an occasional large difference is to be expected.

(g) Conclusions

M. persicae showed a good correlation between sap and virus transmission. Effects of both pre-access fast and access-probe durations were similar for sap and virus transmission and this suggests that they may represent the same phenomenon. Although slightly larger differences were found for H. lactucae, the correlation was still adequate. The overall comparisons of virus and tracer transmission are shown in Fig 16 & 17 (p. 65) for the four experiments.

Table 13. Comparison between numbers of H. lactucae that transmitted more than 7.25 ccpm (= 841 μ^3) and the numbers that transmitted virus for experiment IV

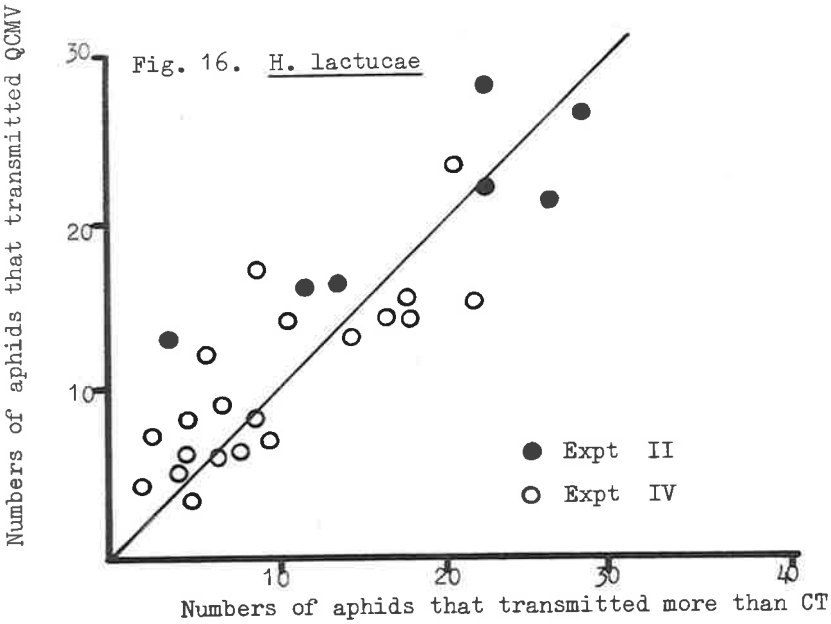
Duration of access-probe (min)	Duration of pre-access fast (hr) 0	1	2	3	Totals
1	4 : 2	7 : 3	8 : 9	7 : 10	26 : 24
3	8 : 5	5 : 4	13 : 15	12 : 6	38 : 30
5	6 : 8	17 : 9	23 : 21	14 : 11	60 : 49
7	6 : 5	3 : 5	15 : 22	15 : 18	39 : 50
9	7 : 9	6 : 7	14 : 17	14 : 18	41 : 51
Totals	31 : 29	38 : 28	73 : 84	62 : 63	204 : 204

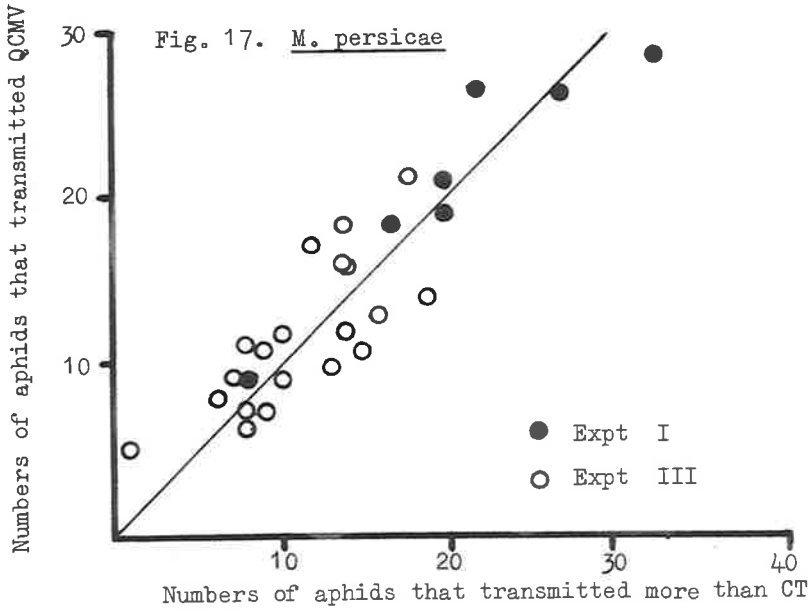
Footnote

(a) The results are expressed as the ratio :-

Transmission of virus : transmission of tracer

Fig. 16 & 17. Tracer and virus transmission for M. persicae and H. lactucae.





DEPENDENCE OF TRANSMISSION ON PREVIOUS APHID BEHAVIOUR

Introduction

In the experiment described in the previous chapter, sap and virus transmission were found to be affected to the same extent by two factors and for two aphid species. It was suggested (p. 63) that sap and virus transmission represent the same phenomenon. The correlation could have been tested further by subjecting aphids to other treatments: for example, post-access fasting, different temperatures, or different access- or inoculation-plants. However, correlations only show likely relationships and so evidence that virus could survive in the cibarium was sought by other means.

One approach that was considered was to study the transmission of viruses to several successive test-plants using single aphids. Experiments of this type were originally done to show that the viruses do not persist in the aphid (Watson, 1938; Watson & Roberts, 1939). However, they raise other issues also, which are more difficult to interpret or explain. For example, in this type of experiment it is not known whether:

(1) aphids acquire similar amounts of virus;

(2) aphids that fail to transmit ever acquired the virus;

(3) transmissions are or are not independent events;

(4) transmission frequencies decrease with successive probes because (a) virus is inactivated in the aphid, (b) virus

is lost from the aphid, or (c) aphid behaviour changes.

Virus transmission to successive test-plants

(a) Experimental methods

Groups of 10 M. persicae that had fasted for 2 hr were allowed a 5-min access-probe on a QCMV-infected plant. In each group, two of the aphids that satisfied the conditions required of probing aphids (p. 17) were retained. Each was allowed to probe 8 successive cucumber seedlings which were grown for symptom assessment. Aphids which became damaged or apparently weakened before the 8th probe were rejected with the seedlings they probed. The process was repeated until 220 aphids had been tested.

The results of two experiments previously published by others are also examined: McLean (1959) used M. persicae to transmit potato feathery mottle virus (PFMV) to 10 successive test-plants, and Sylvester (1955) used the same species to transmit lettuce mosaic virus (LMV) to as many as 13 successive plants. In McLean's experiment, aphids made 15-sec access-probes and 10-sec inoculation-probes, whereas Sylvester allowed aphids 30-sec access-probes and 15 to 45-sec inoculation-probes. Other experiments of this type published by Watson (1938) and Bradley (1952) are not considered because the numbers of aphids they used were too small for the analyses presented here.

(b) Results

M. persicae transmitted QCMV in a non-persistent manner (Table 14, p. 69). Fifty-four percent transmitted virus to the first test-seedling and the frequency of transmission decreased to 7.7% for the eigth seedling. Eighty-one percent of the aphids transmitted to at least one seedling and only 19% failed to transmit to any seedling.

Table 15 (p. 71) shows the number of aphids that transmitted 0, 1, 2..... times and these are compared with the expected numbers calculated from the data in Table 14. Expected numbers were not calculated by the methods used by Bradley (1952), Sylvester (1955) and McLean (1959). In those studies it was assumed that each seedling had the same chance of becoming infected, but the data show that successive seedlings are less likely to become infected. Therefore, an alternative method was used.

The probability of transmission to each of the 8 seedlings (P_i ; i = 1, 2,.... 8) is given in Table 14. For each possible outcome of transmission by an aphid to 8 test-plants, the probability of its occurrence was derived by substituting P_i for transmission and $1 - P_i$ for non-transmission, then multiplying all terms. The outcomes were classified according to the numbers of transmissions they contained, summed to give the corresponding probabilities, and multiplied by the total number of aphids in the experiment to give the expected frequencies. These are tabulated (Table 15)

Table 14. Non-persistence of QCMV in M. persicae during transmission to a series of eight seedlings by single aphids.

Transmission (+) or not (-) to seedlings									
1	2	3	4	5	6	7	8	R	N
+	+	+	+	+	-	+	-	1	6
+	+	+	+	+	-	-	-	1	5
+	+	+	+	-	+	-	-	1	5
+	+	+	+	-	-	-	-	3	4
+	+	+	-	+	-	+	-	2	5
+	+	+	-	+	-	-	-	3	4
+	+	+	-	-	-	-	+	1	4
+	+	+	-	-	-	-	-	12	3
+	+	-	+	-	+	-	+	1	5
+	+	-	+	-	-	-	-	4	3
+	+	-	-	+	+	-	-	1	4
+	+	-	-	+	-	-	-	4	3
+	+	-	-	-	+	-	+	1	4
+	+	-	-	-	-	+	-	1	3
+	+	-	-	-	-	-	-	17	2
+	-	+	+	+	-	+	-	1	5
+	-	+	+	-	+	-	-	1	4
+	-	+	+	-	-	-	-	4	3
+	-	+	-	+	+	-	-	1	4
+	-	+	-	+	-	+	-	2	4
+	-	+	-	-	+	+	-	1	4
+	-	+	-	-	+	-	+	4	4
+	-	+	-	-	-	-	-	6	3
+	-	-	+	+	+	-	-	1	4
+	-	-	+	+	-	-	-	2	3
+	-	-	+	-	+	+	-	1	4
+	-	-	+	-	+	-	+	1	4
+	-	-	+	-	+	-	-	1	3
+	-	-	+	-	-	+	-	2	3
+	-	-	+	-	-	-	+	1	3
+	-	-	+	-	-	-	-	1	2
+	-	-	-	+	-	+	-	1	3
+	-	-	-	+	-	-	-	2	2
+	-	-	-	-	+	-	+	1	3
+	-	-	-	-	+	-	-	1	2
+	-	-	-	-	-	+	-	7	2
+	-	-	-	-	-	-	-	24	1

Table continued opposite ....

Table 14. Continued...

1	2	3	4	5	6	7	8	R	N
-	+	+	+	+	-	+	-	1	5
-	+	+	+	-	-	+	-	1	4
-	+	+	+	-	-	-	-	8	3
-	+	+	-	+	+	-	-	1	4
-	+	+	-	+	-	+	-	1	4
-	+	+	-	+	-	-	-	1	3
-	+	+	-	-	+	+	-	1	4
-	+	+	-	-	+	-	+	1	4
-	+	+	-	-	+	-	-	2	3
-	+	+	-	-	-	-	-	3	2
-	+	-	+	+	+	-	-	1	4
-	+	-	-	+	-	+	-	1	3
-	+	-	-	+	-	-	-	2	2
-	+	-	-	-	-	-	-	3	1
-	-	+	+	+	-	-	-	1	3
-	-	+	+	-	-	-	-	1	2
-	-	+	-	+	+	-	-	1	3
-	-	+	-	+	-	+	-	4	3
-	-	+	-	-	+	+	-	1	3
-	-	+	-	-	+	-	+	2	3
-	-	+	-	-	+	-	-	3	2
-	-	+	-	-	-	-	+	2	2
-	-	+	-	-	-	-	-	3	1
-	-	-	+	+	-	+	-	1	3
-	-	-	+	+	-	-	-	1	2
-	-	-	+	-	+	-	-	3	2
-	-	-	+	-	-	+	-	3	2
-	-	-	+	-	-	-	+	1	2
-	-	-	+	-	-	-	-	1	1
-	-	-	-	-	+	+	-	1	2
-	-	-	-	-	+	-	-	1	1
-	-	-	-	-	-	+	-	1	1
-	-	-	-	-	-	-	-	41	0

$\Sigma+$	119	80	82	50	38	35	36	16
$\Sigma-$	101	140	138	170	182	185	184	204
P_i	.54	.36	.37	.23	.17	.16	.16	.07

Chi-square (7 df) = 174
P <<< 0.001

R is the number of aphids that gave the same result.

N is the number of transmissions per aphid.

P_i is the proportion of seedlings that became infected.

according to two hypotheses:

(1) Aphids which failed to transmit to any seedling did not acquire virus and should therefore be excluded;

(2) All aphids acquired virus and all should be included.

The comparison of observed with expected frequencies shows better agreement for hypothesis (1) for which chi-square is 4.15 (4 df) and $P > 0.3$, than for hypothesis (2) for which chi-square is 29 (5 df) and $P < 0.001$. The method used to derive expected frequencies assumes that the aphids carried similar amounts of virus, that each transmission is an independent event, and that aphid behaviour does not change during the experiment. The experiment shows either that these assumptions are valid (except that some aphids did not acquire virus), or that if all aphids acquired similar amounts of virus then one or more of the other assumptions was seriously violated. The comparisons do not indicate which conclusion is true.

Other comparisons can be examined to test assumptions that may be violated if all aphids are considered to have acquired virus. For example, in Table 16 (p. 72) all test-seedlings are classified according to whether they became infected or not, and whether or not the preceeding seedling became infected. The values of chi-square for each of the seven 2 x 2 contingency tables are shown. Transmissions are shown to be interdependent for the 1st., 2nd.

Table 15. Comparison of observed and expected numbers of aphids that transmitted 0, 1, 2..... times for two hypotheses.

Transmissions per aphid	0	1	2	3	4	5 or mo
Observed	43	33	48	60	28	8
Expected (hypothesis 1)	-	34	59	54	25	7
Expected (hypothesis 2)	19	59	66	48	21	7

The method of deriving the expected numbers of aphids is described on p. 64.

Table 16. Classification of aphids according to transmission result and transmission to the pre-ceeding seedling.

Transmission result (a)	Comparison between seedlings :- 1:2	2:3	3:4	4:5	5:6	6:7	7:8
+:+	53	44	27	11	6	5	0
+:-	66	36	57	41	32	30	35
-:+	27	40	25	27	29	30	17
-:-	74	100	111	141	153	155	168
Chi-square (1 df)	7.5	15.	5.5	0.7	0	0.1	3.5
P	<0.01	<0.01	<0.02	>0.3		>0.7	>0.05
Expected (+:+)	43.2	30.5	19.8	9.2	6.0	5.6	2.7

Footnotes

(a) The four possible results of transmission to two successive seedlings.

and 3rd. comparisons, and possibly also for the 7th. This effect could arise if transmissions were associative. The last row of Table 16 shows the numbers of aphids expected to transmit to both seedlings if transmissions were independent. They were derived in the usual way for the 2 x 2 contingency table. For the first comparison:

$$\text{Expected number} = \frac{(\text{transmission to 1}) \times (\text{transmission to 2})}{(\text{total number of aphids})}$$

$$= \frac{(53 + 66) \times (53 + 27)}{220}$$

$$= 43.3$$

The comparison of these numbers with those observed in the first row of the table shows a systematic trend. Transmissions are more likely if the aphid transmitted to the preceeding seedling than if it did not, but the effect is more marked for early than late transmissions.

A similar test of interdependence can be applied by considering transmissions to be either early or late (Table 17, p. 74). In the first comparison, aphids are classified according to whether they transmitted to the first seedling and whether they transmitted to any other seedling. In the second comparison, they are reclassified according to transmissions to the first or second seedling, and transmission to any of the remaining six seedlings. Aphids transmitted late more often when they also transmitted

Table 17. Five comparisons of early and late transmissions of QCMV by M. persicae.

	No. of early seedlings	1	2	3	4	5
	No. of late seedlings	7	6	5	4	3
Transmission results						
Early	Late					
+	+	53	95	102	98	91
+	-	66	24	44	68	85
-	+	27	30	33	13	3
-	-	74	41	41	41	41
Chi-square (1df)		7.5	27	13	20	29
P < 0.01						

early, irrespective of the number of seedlings classed as early or late.

The general conclusion from Tables 16 & 17 is that transmissions are associative; a corollary of this is that failures to transmit are associative. Two different ways can cause non-transmissions to be associative:

(1) some aphids did not acquire virus

(2) when an aphid failed to transmit virus, then changes occurred in the location or infectivity of transmissible virus (or in aphid behaviour) which reduced the chance of subsequent transmission.

Although these Tables help to formulate the nature of possible interdependence of transmissions, the two extreme conclusions remain possible.

The data published by McLean (1959) for the transmission of PFMV (Table 18, p.76) show similar irregularities. Transmissions can be considered independent events only if it is assumed that aphids which did not transmit virus, did not acquire it (Tables 19 & 20, pp. 77 & 78).

Somewhat different results were obtained by Sylvester (1955) for the transmission of LMV by M. persicae (Table 22, p.79). It was not necessary to assume that any of 102 aphids, which did not transmit virus, had never acquired it (Table 22, p.80). Also, although the number of transmissions was low, several aphids transmitted

Table 18. Results of McLean (1959) for the transmission of PFMV by *M. persicae* to 10 successive seedlings.

Test-plants											
1	2	3	4	5	6	7	8	9	10	R	N
+	+	-	+	-	+	+	-	-	-	1	5
+	+	-	+	-	+	-	-	-	-	1	4
+	+	-	+	-	-	-	+	-	-	2	4
+	+	-	o	+	-	+	-	+	-	1	5
+	+	-	-	-	-	+	-	-	-	1	3
+	+	-	-	-	-	-	+	-	-	1	3
+	+	-	-	-	-	-	-	-	+	1	3
+	-	+	+	-	-	-	+	-	-	2	4
+	-	+	-	+	+	-	-	-	-	1	4
+	-	+	-	+	-	+	+	-	-	1	5
+	-	+	-	+	-	-	-	-	-	1	3
+	-	+	-	-	+	-	-	+	+	1	5
+	-	+	-	-	+	-	-	+	-	1	4
+	-	-	+	-	-	-	+	-	-	1	3
+	-	-	+	-	-	-	-	-	-	3	2
+	-	-	-	+	-	+	-	-	-	1	3
+	-	-	-	-	+	-	-	-	-	1	2
+	-	-	-	-	-	-	+	-	-	1	2
+	-	-	-	-	-	-	-	-	-	3	1
-	+	+	-	+	-	+	-	-	-	1	4
-	+	+	-	+	-	-	-	-	-	4	3
-	+	+	-	-	-	+	-	-	-	1	3
-	+	+	-	-	-	-	-	-	+	1	3
-	+	-	+	+	+	-	-	-	-	1	4
-	+	-	+	-	-	+	-	-	-	1	3
-	+	-	-	+	-	-	-	+	-	1	3
-	+	-	-	+	-	-	-	-	-	1	2
-	+	-	-	-	-	-	-	-	-	2	1
-	-	-	-	-	-	-	-	-	-	20	0
25	21	13	12	13	7	8	8	4	3	Chi-square (9df) = 49	
33	37	45	45	45	51	50	50	54	55	P <<< 0.001	

o means seedling died
R is the number of aphids that gave the same result.
N is the number of transmissions per aphid.

Table 19. Numbers of M. persicae that transmitted PFMV 0, 1, 2.... times to a series of 10 test-plants.

Transmissions per aphid	0	1	2	3	4	5 or more
Observed	20	5	6	14	9	4
Expected (Hypothesis 1)	-	4.7	9.1	11.1	8	5
Expected (Hypothesis 2)	3	10.8	15.6	12.3	5.9	2.6

The method of deriving the expected numbers of aphids is described on p. 68

Table 20. Comparisons of early and late transmissions of PFMV by *M. persicae*. (Data from McLean, 1959).

No. early seedlings		1	2	3	4	5	6	7	8
No. late aeedlings		9	8	7	6	5	4	3	2
Transmission results									
Early seedlings	Late seedlings								
+	+	8	33	33	30	24	20	14	6
+	-	17	5	5	8	14	18	24	32
-	+	13	0	0	0	0	0	0	0
-	-	12	12	12	12	12	12	12	12
Chi-square (1df)		2.05	31	31	22	16	6.8	2.74	2.0
Expected (+:+)		10.5	25	25	23	18	15	10	4.6

Table 21. Results of Sylvester (1955) for the transmission of LMV by M. persicae to successive test-plants.

	Test-plants.															
	1	2	3	4	5	6	7	8	9	10	11	12	13	R	N	
	+	-	-											1	1	
	+	-	-	-	-	-	-							2	1	
	+	-	-	-	-	-	-	-	-	-	-	-		1	1	
	+	-	-	-	-	-	-	-	-	-	-	-	-	1	1	
	+	-	-	-	-	-	-	-	-	-	-	-	-	1	1	o
	+	+	+	+	-	-	-	-	-	-	-	-		1	4	
	+	-	+	-	-	-	-	-	-	-				1	2	
	+	-	-	+	-	-	-	-	-	-	-	-	-	1	2	
	-	+	-	-	-	-	-							1	1	
	-	+	-	-	-	-	-	-						1	1	
	-	+	-	-	-	-	-	-	-	-	-	-	-	1	1	
	-	+	-	-	-	-	-	-	-	-	-	-	-	4	1	o
	-	+	-	+	-	-	-	-	-	-	-	-	-	1	2	
	-	-	+	-	-	-	-	-						1	1	
	-	-	+	-	-	-	-	-	-	-				1	1	
	-	-	+	-	-	-	-	-	-	-	-	-	-	2	1	
	-	-	+	+	-	+	-	-						1	3	
	-	-	-	+	-	-	-	-	-	-	-			1	1	
	-	-	-	+	-	-	-	-	-	-	-	-		1	1	
	-	-	-	+	-	-	-	-	-	-	-	-	-	1	1	
	-	-	-	+	-	-	-	+	-	-	-	-	-	1	2	
	-	-	-	-	+	-	-	-	-					1	1	
	-	-	-	-	+	-	-	-	-	-	-	-	-	2	1	
	-	-	-	-	-	-	+	-	-	-	-	-	-	2	1	
	-	-	-	-	-	-	-	-	+	+	-	-	-	1	2	
	-	-	-	-	-	-	-	-	-	+	+	-	-	1	2	
	-	-	-	-	-	-	-	-	-	-	-	-	+	1	1	
	-	-	-	-	-	-	-	-	-	-	-	-	-	102	0	
Σ+	9	9	7	8	3	1	2	1	1	2	1	0	1			
Σ-	127	127	129	127	132	134	133	131	128	126	126	125	121			

R = number of aphids that gave the same result.

N is number of times each aphid transmitted virus.

o means that these aphids were allowed additional probes, none of which resulted in transmission of LMV.

Table 22. Numbers of M. persicae that transmitted LMV 0, 1, 2.... times to a series of 13 test-plants.

Transmissions per aphid:	0	1	2	3 or more
Observed	102	26	6	2
Expected (hypothesis 2)	99	33	4.5	0.3

The method of deriving the expected frequencies is described on page 68. The meaning of hypothesis 2 is explained on page 70.

for the first time while making their 5th or later probes. In Table 23 (p. 82), transmissions to successive pairs of plants are shown. Expected frequencies of associated transmissions are low and chi-square is poorly estimated. Nevertheless, associative transmissions are suspiciously common. In this respect, Sylveter's experiment with LMV is similar to those with PFMV (McLean, 1959) and QCMV described above.

(c) Conclusions

It may be considered unwise to attempt to reconcile three experiments carried out by different people, using different viruses, plants aphid strains and experimental methods. However, such a reconciliation is of interest in relation to the location of transmissible virus and leads to an explanation which can be tested by further experimentation.

The general conclusion for the results obtained with QCMV and McLean's data for PFMV transmission (p. 75) was that transmissions may not be independent events; that failures to transmit may be associative. This conclusion is applicable also to the results obtained by Sylvester for the transmission of LMV (p. 75). Associative failures could arise because many aphids fail to acquire virus, but could also arise because either the location or infectivity of transmissible virus changes when aphids fail to transmit virus.

Table 23. Classification of aphids according to transmission results to two successive test-seedlings.

Transmission result (a)	Comparisons between test-seedlings :-										
	1:2	2:3	3:4	4:5	5:6	6:7	7:8	8:9	9:10	10:11	Totals
+:+	1	1	2	0	0	0	0	0	1	1	6
+:-	8	8	5	8	3	1	2	1	0	1	37
-:+	8	6	6	3	1	2	1	1	1	0	29
-:-	119	121	123	124	131	132	129	127	126	124	1255
Expected (+:+)	.6	.46	.41	.18	.022	.015	.015	.008	.016	.016	1.742

Footnotes

(a) The four possible results of transmission to two successive seedlings.

The expected numbers are derived as explained on page 72.

The 10 comparisons are independent.

The volumes of sap imbibed during access-probes are large in relation to the dimensions of the mouthparts (p. 27). Similar amounts may be imbibed during inoculation-probes; that is, there may be no fundamental difference in aphid behaviour during access- and inoculation -probes. The three experiments could be reconciled if it could be shown that failure to transmit is enhanced by sap-uptake from the healthy seedling, and that the imbibed sap could displace or dilute virus held within the aphid. This would explain:

(1) Successful virus transmissions are associative for early inoculation-probes, when aphids had not imbibed much healthy sap;

(2) Failures to transmit would be associative when aphids imbibed large amounts of sap during inoculation-probes;

(3) Some aphids that had often failed to transmit virus may transmit it after transmission of the healthy sap they carried;

(4) Non-persistence could be explained by the increasing chances of virus migration into the stomach, dilution of virus by healthy sap imbibed into the cibarium, or loss by transmission.

The assumption that sap-uptake can occur during inoculation-probes, as during access-probes, is considered reasonable. However, independent evidence is required that transmissible virus can be

displaced in the cibarium but later transmitted, and that this could cause non-randomness of transmissions.

Transmission of two viruses acquired during successive probes

Groups of 10 M. persicae were collected and fasted for 2hr. The aphids were allowed two successive access-probes of 5-min and 3-min duration on two different plants, then a single inoculation-probe lasting 1 min on healthy Nicotiana glutinosa seedlings. The two access-probes were either:

Sequence 1. Cucumber infected with QCMV then Physalis floridana infected with PVY

Sequence 2 Cucumber infected with QCMV then healthy P. floridana

Sequence 3 Healthy cucumber then P. floridana infected with PVY

Sequence 4 P. floridana infected with PVY then cucumber infected with QCMV

Sequence 5 Healthy P. floridana then cucumber infected with QCMV

Sequence 6 P. floridana infected with PVY then healthy cucumber.

The first access-probe was always the longer and was optimum for virus acquisition with the aphids used. The second access-probe was sub-optimal and was chosen to reduce the chance that aphids would lose completely their ability to transmit the first virus. Groups of aphids were assigned to the sequences in random order, and test-plants were grown for symptom assessment. Plants from sequences 1 and 4 (which could become infected with one or both viruses)

were assayed for QCMV by sap-inoculation to cucumber and for PVY by sap-inoculation to P. floridana, only if they showed symptoms. Test-plants from other sequences were examined only for symptoms.

When aphids made access-probes on two diseased plants (sequences 1 and 4), transmission of the two viruses was not independent (Table 24, p. 86). In both sequences, the transmission of the first virus was low when aphids also transmitted the second virus. Sequences 1 and 4 can be combined in two ways, to maintain either the sequences or the two viruses as the basis of classifying the aphids. When they are combined according to the transmission of QCMV and PVY, ignoring the order of the access-probes (Table 25a, p. 87) the classification represents the effect of one virus on the transmission of the other, or the susceptibility of the host-plants, or the detection of one virus in the presence of the other. If combined to retain the order of acquisition, ignoring the viruses used (Table 25b), then the classification shows the effect of one access-probe on the other. Both methods show that the factors used to classify the aphids are not independent. However, the two methods of combining sequences 1 and 4 are not independent of each other and, although they represent different phenomena, interdependence between one pair of factors is not separated from interdependence of the other pair.

If the two viruses interfere with each other, rather than the sequences in which they were acquired, then aphids that probed

Table 24. Interaction of PVY and QCMV transmission by single *M. persicae*.

Sequence (1) QCMV acquired first.

		Transmission of PVY	
		+	-
Transmission	+	7	11
of QCMV	-	38	18

Chi-square (1 df) = 4.8; $P < 0.05$

Sequence (4) PVY acquired first.

		Transmission of QCMV	
		+	-
Transmission	+	5	33
of PVY	-	31	16

Chi-square (1 df) = 14.1 ; $P < 0.01$

Table 25. The combination of sequences 1 and 4 (Table 24) so as to retain classification of aphids according to either 1st and 2nd virus (a) or PVY and QCMV (b).

(a)

		Transmission of 2nd Virus	
		+	-
Transmission of	+	12	44
1st virus	-	69	34

Chi-square (1 df) = 28.2: $P < 0.001$

(b)

		Transmission of PVY	
		+	-
Transmission of	+	12	42
QCMV	-	71	34

Chi-square (1 df) = 28.8; $P < 0.001$

two virus infected plants should transmit one or both viruses less often than aphids which probed only one infected plant. The information for testing this is supplied by comparisons between the other sequences; the transmission of QCMV by aphids that probed also on plants infected with PVY or not (Table 26, p. 89), or the transmission of PVY by aphids that did or did not probe on plants infected with QCMV (Table 27, p. 90). The comparisons show that the acquisition of virus did not have an effect different from the acquisition of healthy sap. The effects shown in Tables 24 (p. 86) and 25 (p.87) are therefore caused by virus displacement within the aphid, or its replacement on or in the stylets, by the sap imbibed during the second probe.

The alternatives were resolved in the following experiment. Groups of 10 M. persicae were fasted for two hours then allowed to make four probes:

(1) a 5-min probe on P. floridana infected with PVY;

(2) a 3-min probe on cucumber infected with QCMV;

(3) a 1-min inoculation-probe on healthy P. floridana;

(4) a 1-min inoculation-probe on healthy N. glutinosa; aphids were left to probe or feed on this plant overnight.

Only the one sequence of four probes was used. The test-plants were grown for symptom assessment, and the N. glutinosa seedlings which showed symptoms were assayed for QCMV and PVY by sap-inoculation to cucumber and P. floridana.

Table 26. Transmission of QCMV by aphids that probed PVY infected or healthy plants after (a) or before (b) they probed a QCMV infected plant.

(a) QCMV acquired first; comparison of sequences 1 and 2.

	QCMV transmission	
	+	-
Second probe on:		
(1) Infected P. floridana	18	56
(2) Healthy P. floridana	11	26

Chi-square (1 df) = 0.2; P > 0.7

(b) QCMV acquired second; comparison of sequences 4 and 5.

	QCMV transmission	
	+	-
First probe on:		
(4) Infected P. floridana	36	49
(5) Healthy P. floridana	11	36

Chi-square (1 df) = 2.3; P > 0.1

Table 27. Transmission of PVY by aphids that probed QCMV infected or healthy plants after (a) or before (b) they probed PVY infected plants.

(a) PVY acquired first; comparison of PVY transmission in sequences 4 and 6

	PVY transmission	
	+	-
Second probe on;		
(6) healthy cucumber	16	27
(4) infected cucumber	38	47

Chi-square (1 df) = 2.3; $P > 0.1$

(b) PVY acquired second; comparison of sequences 1 and 3

	PVY transmission	
	+	-
First probe on:		
(1) infected cucumber	45	29
(3) healthy cucumber	26	17

Chi-square (1 df) = 0.1; $P > 0.9$

Considering only the aphids that transmitted PVY to at least one seedling, and were therefore known to carry PVY, the aphids transmitted PVY to P. floridana (Table 28,p.92) less often when they carried QCMV (4/22) than when they did not carry QCMV (23/42). Presumably, QCMV displaced transmissible PVY in the aphid, but some aphids could transmit the PVY so displaced. Displacement by QCMV did not completely prevent PVY transmission and this may indicate that mixing of the two viruses can occur in the cibarium

Table 28. Delayed transmission of PVY when aphids retained QCMV they had acquired in a second probe.

Transmission result for		Numbers of aphids (a)
PVY to 1st. healthy seedling.	QCMV to 2nd. healthy seedling.	
+	+	4
+	-	23
-	+	18
-	-	19
Chi-square (1df)		4.60
P		< 0.05

Footnote

(a) Only aphids that transmitted to either the first or second seedling are known to have acquired PVY and only these are included in the table.

23 additional aphids transmitted QCMV but not PVY.

34 aphids did not transmit either virus.

GENERAL DISCUSSION

Virus transmission by sap-uptake and ejection

An alternative to the hypothesis of stylet contamination is proposed on the basis of sap-uptake and ejection described in this thesis.

When aphids imbibe and eject sap from infected plants, they also imbibe and eject virus. This is shown by the generally good agreement between frequencies of sap and virus transmission (Fig. 16 & 17, p. 65). The hypothesis allows estimation of the amounts of sap aphids probably transmit when they transmit virus and these are sufficiently consistent to show how pre-access fasting and access-probe duration affect virus transmission. The intermittent transmission of sap by aphids (Table 6, p. 35) corresponds with the serial transmission experiments done with QCMV and with similar experiments done by Sylvester (1955) and McLean (1959). The location of transmissible virus in the cibarium provides an explanation of the way aphids may retain virus for several probes without transmitting, and without excessive virus loss. The realisation that inoculation probes are essentially the same as access-probes, and that sap-uptake can occur during either, is of particular importance. This can explain non-randomness in serial transmission experiments (p. 81), virus displacement in aphids (p.91), and the usually low frequencies

of transmission of these viruses (usually less than 0.7). Also, because prolonged probing on a test-plant is likely to favour sap-uptake, virus may eventually be displaced to places from which it cannot be transmitted (e.g. the stomach). This could explain why more aphids can be shown to transmit virus when they make several brief inoculation probes than when they make only one long one (Watson & Roberts, 1940). Aphids may cease to be able to transmit non-persistent virus after probing a test-plant for 15 to 20 min because this is the time generally taken by aphids to reach the phloem (Roberts, 1940), and the large amounts of sap ingested could flush the anterior parts of the alimentary canal free of virus.

Non-persistence can be explained in two ways, without involving virus inactivation in or on the stylets. Virus can be displaced in the ways described above so that successive inoculation-probes require the transmission of larger amounts of healthy sap. In addition, aphids may imbibe saliva even when they are not probing plants and this may displace virus in the aphid. However, some aphids transmit large proportions of the sap they carry (p. 33). In the absence of virus multiplication in the aphids, virus transmission must reduce the number of transmissions the aphid can subsequently make. Thus, as the result of either displacement of virus in the aphid, or loss of virus by transmission, transmission frequencies are likely to

decrease after access-probes, and more so for feeding than for fasting aphids.

Whereas the evidence for stylet contamination is based on treatments which reduce virus transmission, and could do so in many ways (p. 6), virus transmission by sap imbibition and ejection is based on evidence of a positive nature - the amounts of sap involved and their distribution in the aphid - which is compatible with a wide variety of features of non-persistent virus transmission. Although the results and ideas in this thesis are largely compatible with the results published by others, there are some important differences in both fact and opinion.

Pre-access fasting and access-probe duration

Single aphids could not be used for simultaneous sap and virus transmission. It was therefore necessary to use the effects of pre-access fasting and access-probe duration to provide groups of aphids for which sap and virus transmission could be compared. These treatments have been extensively used by others to obtain optimum transmission rates. However, they have often been reported to have different effects on transmission than those described in this thesis.

For example, McLean (1959) and Bradley (1961) found that about 15 min pre-access fasting was sufficient for optimum transmission. Bradley (1959, 1964) suggested that pre-access

fasting affects aphid behaviour rather than plays an important part in the transmission mechanism. Also, optimum transmission frequencies are often obtained with access-probes lasting only 15 sec (McLean, 1959) and generally with probes lasting less than 3 min (Watson & Roberts, 1939; Bradley & Rideout, 1953; Swenson, 1960). These time intervals are shorter than those found optimum for QCMV transmission by either M. persicae or H. lactucae. However, the decrease in transmission frequency after prolonged probes found for QCMV transmission, is in agreement with that found by others (Watson & Roberts, 1939; Bradley, 1954; Sylvester, 1954).

These differences may only reflect differences in technique or variation between different aphid species or strains. The experimental designs used may also be important. Watson & Roberts (1939) found that an effect of pre-access fasting could be detected for aphids that made 2-min access-probes, but that the effect decreased for longer probes. My results are similar, but in addition the effect of pre-access fasting is less marked when aphids make brief probes as well as when they make prolonged ones. Because of this interaction between the factors, an effect of access-probe duration could not be detected for unfasted aphids, and an effect of pre-access fasting could not be detected for access-probes that lasted for only one minute. The detection of these effects requires the use of factorial designs, or similar ones

in which both factors are varied, such as those used by Watson & Roberts (1939) and those used in this thesis. This is not always done (McLean, 1959) and may explain some of the differences in the results obtained. Alternatively, efficiency of transmission may affect the ability to detect increases in the amount of virus carried even after brief probes (Bradley, 1964) and possibly similar increases caused by pre-access fasting.

Aphids may vary greatly in their response to these kinds of treatments and different techniques may show different effects. However, the critical feature of my hypothesis for virus transmission by sap-imbibition and ejection, is not whether or not fasting or access-probe duration affect transmission but that if they do, sap and virus transmission should be similarly affected. M. persicae and H. lactucae were both affected by these treatments, in similar ways for sap and virus transmission. The treatments could not be shown to affect sap or virus transmission by M. euphorbiae and A. solani because of the low frequencies of both sap-uptake and virus transmission. With species such as M. euphorbiae and A. solani , and variable species such as A. gossypii and A. craccivora, correlations between sap and virus transmission are ineffective. For these species, a method of measuring sap and virus transmission for single aphids would be most valuable, but such experiments were not feasible.

Non-persistence of virus in aphids

The explanation of non-persistence given on page 94 considers transmissible virus carried in the cibarium and stylets. Virus may be lost from these parts by migration or displacement backwards towards the stomach (from which regurgitation would probably be restricted by the oesophageal valve), by dilution in the cibarium or stylets, or by transmission. Nishi (1963) suggested that non-persistence depended on virus inactivation in the aphid. The for this was based on a comparison of virus transmission with tracer transmission measured by autoradiography. Aphids retained their ability to transmit tracer when fasted for 6 hr after the access-probe but similar aphids lost the ability to transmit virus when fasted for 3 to 5 hr. In this experiment, access-probes were unusually long (10 min, 1 hr, or 24 hr) for the transmission of non-persistent viruses. Nishi (1963) also compared sap and tracer transmission by aphids that made 30 sec access-probes then up to 20 successive inoculation-probes to test-plants. Although the aphids did not transmit virus after the 8th probe, similar aphids transmitted tracer to even the 20th seedling. The mechanism of tracer transmission was not studied; and experiments with simultaneous transmission of tracer and virus were not done presumably because tracer assay (by autoradiography) required the destruction of the test-seedlings. However, it was shown that aphid saliva could inactivate the virus (turnip mosaic virus) used in the transmission

experiments, and Nishi concluded that the loss of virus, but not tracer, was because only the virus could be inactivated by the saliva.

There are several alternative explanations for the prolonged transmission of tracer observed by Nishi (1963). Autoradiography is probably more sensitive for tracer assay than virus transmission is for measuring the amount of virus in aphids. Sap-volumes too small to cause infection may still contain sufficient tracer for detection. Furthermore, the experiments were done using either long access-probes or a long series of inoculation-probes that lasted 40 to 80 min. It is likely that, under these conditions, many or all aphids would have ingested tracer which could then be metabolised and transmitted with saliva for many more probes than virus, which could not be transmitted in this way. It is for these reasons that access-probes were not prolonged in the experiments described on pages 21 to 65 , and that prolonged serial transmission experiments were not done with aphids from labelled plants.

The inactivation of viruses by aphid secretions is particularly important in relation to specificity and these aspects of Nishi's experiments are discussed in the next section.

Specificity and virus inactivation in aphids

Some aphid species are more usually efficient vectors of non-persistent viruses than other species. It was suggested (p.97) that differences in aphid behaviour, in particular sap-uptake and ejection while probing, may contribute to specificity. However, this is unlikely to explain several examples, especially the non-transmission of tobacco mosaic virus (TMV), potato virus X (PVX), turnip yellow mosaic virus (TYMV) and others by probing aphids. Several attempts have been made to assign transmission failures to virus inactivation by aphid saliva. Day & Irzykiewicz (1954) reported that saliva from two plant bugs, neither of which is known to transmit non-persistent viruses, inactivated turnip mosaic virus (TuMV) more strongly than it inactivated TMV. Experiments with aphid saliva were not done.

On the other hand, Nishi (1963, 1969) and Nakazawa (1965) have tested aphid saliva agsinst two non-persistent viruses (CMV and TuMV) and against TMV and PVX and have found specificity in virus inactivation. Two techniques have been used to collect the saliva. Nishi (1958, 1963, 1969) allowed aphids to probe and feed on small amounts of leaf tissuewhich were homogenised and shown to have marked anti-viral effects relative to sap from plants that had not been infested with aphids. Saliva was also collected by allowing aphids to probe sucrose solutions which were then shown to reduce the infectivity of TMV (Nishi, 1963) and different strains of

CMV (Nakazawa, 1965) relative to untreated sucrose solutions. Partial isolation of an active substance has been achieved, by alcohol precipitation and DEAE cellulose column chromatography, which is thought to be a nucleoprotein (Nishi, 1963,1969). For this work, saliva was collected from extracts of leaves on which aphids had probed, but collections of saliva by the other method would seem to offer more valid conclusions. Damage to plants by virus infection (Sela & Applebaum, 1964), fungal infections (Hodgson & Munro, 1966) and leaf infiltration with bacteria (Loebenstein & Lovrekovich, 1966) can induce resistance in plants and the phenomenon may be widespread. By using plant materials probed by large numbers of aphids, Nishi (1958, 1962 1963, 1969) may have been studying the production of antiviral substances in plants rather than their presence in aphid secretions. This criticism cannot be applied to the other method of collection (Nishi, 1963; Nakazawa, 1965) using sucrose solutions into which aphids had probed.

Although the secretions collected by either method show a large effect on TMV with which they are mixed, they may not be as important to virus in the aphid. The transmission of TMV can, in fact, be aided by aphids and the virus can survive contact with their stylets, and so presumably with aphid secretions also. Orlob (1963) could recover infective TMV from stylets that had been dipped in virus provided that the rostrum had not extended over them.

Aphids inoculated plants with TMV when they probed leaves that had been sprayed with virus (Teakle & Sylvester, 1962); aphids secrete saliva immediately before (Van Hoof, 1958) and during (Lamb, Ehrhardt & Moericke, 1960) probing, and the transmitted TMV probably made contact with the sheath before and during inoculation into the plant. Also, Pirone (1967) showed that aphids could acquire TMV when probing solutions of the virus, and could release infective virus when they inserted their stylets into buffer. Thus the evidence that aphid secretions prevent TMV transmissions from plant to plant may be ill-founded.

Sap-uptake and host selection

The explanation of virus transmission by sap imbibition and ejection raises the questions of the way aphids imbibe sap, how factors affecting transmission operate on sap-uptake, and the role sap-uptake plays in aphid behaviour. The cibarial-pharangeal pump seems well adapted to operate as a peristaltic pump. In this way it could function without hindrance by the oesophageal valve, could cause imbibition and ejection of sap, and may be able to force materials into the epi-pharangeal ducts and so into contact with the overlying hypodermal cells. Sap-uptake may be by several methods, including pharangeal pump activity, cell turgor pressure and capillarity. On the other hand, sap-ejection may operate only by the action of the pharangeal pump.

The uptake of small sap samples by probing aphids may serve to aid host-selection of the type described by Wensler (1960), or discrimination between fluids they probe (Mittler & Dadd, 1962). As a result of sap-sampling, they may achieve stimuli which direct feeding and may be responsible for a gradual change from sap-sampling to phloem-seeking and feeding. This could explain the fall in transmission frequency with long probes. Fasting may enhance sap-sampling by aphids that had fed on the phloem, by allowing such stimuli to decay.

However, aphids vary greatly in their host requirements and in details of their behaviour, and the ideas expressed on host sampling may prove quite inadequate.

Epilogue

The wide acceptance of any hypothesis must await attempts to apply it to other systems, and citical examination of its deficiences. Because of apparent variations among even the six aphid species studied, and variations in technique that are apparent among other studies of non-persistent viruses, exceptions may be many. There is no reason why stylet contamination should not play a part in virus transmission as well as in sap-ejection, and it may be important in some instances with efficient vectors and easily transmitted viruses. Its rejection in this work is because it was not demonstrated (and could not be demonstrated

in the presence of sap-ejection) and because it is insufficient to account for many observations.

Several lines of work may prove useful in deciding whether sap-uptake is generally important. The transmission of purified virus, in the presence of amounts of tracer sufficiently small to allow virus to survive, may allow a direct correlation of tracer and virus transmission with single aphids. This would provide a powerful technique for the study of the transmission mechanism, the amounts of sap transmitted, and the stability of the virus in the aphid. It would also widen the range of aphids that could be used.

In the absence of a method for comparing sap and virus transmission with single aphids, the experiments described in this thesis for the transmission of two viruses acquired in separate probes, may be extended to provide further useful information. Because they do not rely on consistent responses to some treatment, almost any combination of virus and vector could be used provided that transmission frequencies are reasonably high and that the viruses can be recognised together in the test-plants, or separated by assay. The survival of non-persistent viruses in probing aphids and the ease with which they may be displaced (as opposed to replaced) and later transmitted, is probably fundamental to the idea that they are transmitted by sap-uptake into the cibarium and ejection from it.

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