OBSERVATIONS ON THE FEEDING OF THE VIRUS VECTOR *OROSIUS ARGENTATUS* (EVANS), AND COMPARISONS WITH CERTAIN OTHER JASSIDS

By M. F. Day, H. Irzykiewicz, and Anne McKinnon*

Summary

Examination of feeding tracks of several species of jassids in plant petioles shows that each species produces characteristic stylet sheaths. Most species feed in both parenchyma and vascular bundles and they exhibit varying degrees of preference for phloem tissue. An attempt to confirm published observations on a pH gradient towards the phloem failed to reveal such a gradient in many of the plants examined; in fact, it has been demonstrated that no such gradient is required to explain the feeding behaviour of the jassids studied. It is concluded that they find the tissues upon which they feed by random probing with the stylets.

A study of the details of jassid feeding has suggested possible explanations for several puzzling observations on virus transmission by these insects.

I. Introduction

The feeding processes of an insect vector of a virus disease are of obvious significance, for it is during these events that the pathogen is first taken up from one host and subsequently reinoculated into a second susceptible host. The common brown jassid *Orosius argentatus* (Evans) is a vector of several phytopathogenic viruses of importance in Australia; but very little is known of its physiology. This deficiency is the more regrettable because generalizations on jassid feeding have been largely based on the observations made on *Circulifer* (*Eutettix*) *tenellus* (Baker), the vector of curly top of sugar beet (see, for example, Bennett 1935; Bennett and Wallace 1938), and on *Cicadulina mbila* (Naude), vector of maize streak (Storey 1938, and earlier papers quoted therein). That these two species, both mainly phloem feeders, are not representative of the range of vector behaviour has recently been strikingly demonstrated by the discovery that all the vectors of Pierce's disease of grape feed mainly on the xylem (Houston, Esau, and Hewitt 1947). The mechanism whereby *Circulifer tenellus* finds the phloem of the sugar beet has been studied in an ingenious series of experiments by Fife and Frampton (1936). They reported a gradient of increasing alkalinity from the cortex to the vascular bundle amounting to a difference of about 1.6 pH units. Further, they found by feeding an artificial diet that the insects could distinguish between media of different pH and that they preferred an alkaline medium; they con-

* Division of Entomology, C.S.I.R.O., Canberra, A.C.T.

cluded that the jassids find the preferred tissue of the host with the aid of this pH gradient. It has been assumed that this mechanism holds for other jassids but confirmatory evidence has not been published. It is the purpose of this paper, therefore, (1) to report observations on the plant tissues upon which *O. argentatus* and several other jassids feed; (2) to consider the methods by which they find these tissues with reference to the Fife and Frampton pH gradient hypothesis; and (3) to describe observations on mouth-part movements and related details that assist in giving an outline of the feeding behaviour of some vectors and non-vectors of phytopathogenic viruses. All of the jassids were from laboratory colonies originating from insects collected in the vicinity of Canberra, usually by trailing nets from a jeep traversing stands of lucerne.

II. THE TISSUES ON WHICH SEVERAL SPECIES OF JASSIDS FEED

(a) Characteristics of Feeding Tracks of Six Species of Jassids

Jassid feeding tracks are made readily visible in sections of plant tissues by the use of Millon's reagent for tyrosine (Smith 1933). Many sections of plant petioles, stems, or leaves have been cut approximately 150 μ in thickness in a hand microtome and immediately placed in Millon's reagent. After about 10 minutes, jassid feeding tracks are reddish and can be clearly distinguished from the plant tissues. Evidence that the reaction gives an accurate picture of the position of the insect mouth-parts in the plant is given below (Section V). The sections were examined with a dissecting microscope under a magnification of about 40 diameters, and the tracks drawn with the aid of a camera lucida. Numbers of tracks can be found if many insects are exposed for several days on a few petioles, or better, on a restricted portion of a single petiole.

TABLE 1

TISSUES OF *MALVA PARVIFLORA* PETIOLES IN WHICH FEEDING TRACKS OF SEVERAL JASSIDS TERMINATE

Species	Total Tracks Counted	Percentage of Tracks in		
		Phloem	Xylem	Parenchyma
Limotettix sp.	109	63	0	37
Orosius argentatus (Evans)	108	54	3	43
Eurinoscopus punctatus Evans	104	37	8	55
Euscelis sp.	128	37	27	36
Nesoclutha obscura (Evans)	77	5	0	95
Erythroneura ix Myers	20*	5	5	90

* This species did not feed readily on *Malva* and hence it did not prove feasible to obtain a greater number of tracks.

In order to study the tissues preferred for feeding, petioles or stems with collateral vascular bundles without secondary thickening are necessary. Those of the petiole of *Malva parviflora* (L.) have proven very suitable. The results, showing the final tissue reached by several species of jassids, are given in Table 1.

From these data it may be concluded that (1) it is usual to find some feeding tracks from any one species terminating either in vascular bundles or in parenchyma, and (2) species vary in respect to the plant tissues upon which they generally feed.

The second conclusion presupposes some ability of the insect to distinguish tissues. It also suggests that the species studied would vary in their ability to transmit phloem viruses on this characteristic alone. More detailed information was gained from a study of the form of the tracks made by each species.
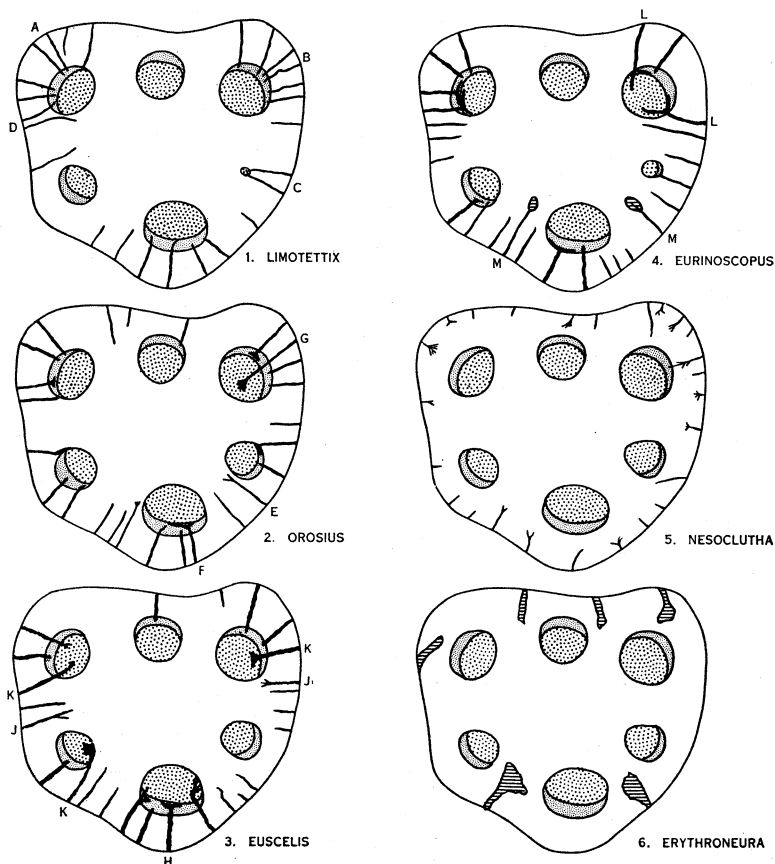
Figs. 1-6.—Diagrams of transverse sections of petioles of *Malva* showing feeding tracks of (1) *Limotettix*, (2) *Orosius*, (3) *Euscelis*, (4) *Eurinoscopus*, (5) *Nesoclutha*, (6) *Erythroneura*. For further details see text.

Diagrams of random sections of *Malva* petioles containing superimposed feeding tracks are presented in Figures 1-6. The following conclusions with respect to each species may be drawn from the data presented in these figures.

(1) *Limotettix*. Not only is there a high percentage of the tracks that terminate in the phloem tissue, but it will be observed that they are concentrated in regions where they will reach the phloem without curving or branch-

ing (Fig. 1, *A* and *B*). There is an absence of tracks from the lateral regions of the petiole, except at points such as *C*, in which example the tracks run almost directly to a very small vascular bundle. There is little curving of the tracks, but at *D* there are two tracks that curve slightly, one in the direction of the phloem, the other away from the vascular bundle.

(2) *Orosius*. The percentage of tracks reaching the phloem is lower than in the previous species, and the tracks are more uniformly distributed around the petiole. Branching occurs in some tracks (*E*) and some slight tissue damage is apparent, especially in those tracks that terminate in the phloem (*F*). These are noticeably larger than tracks terminating in the parenchyma, probably owing to the insect feeding for a longer time on phloem than it does on parenchyma. Occasional tracks terminate in the xylem (*G*).

(3) *Euscelis*. This species produces more tissue damage (*H*) than the damage following feeding by *Orosius*. More tracks terminate in the xylem (*K*), and branching is more frequent (*J*). The tracks are generally larger than those of *Orosius*, probably correlated with the larger size of the jassid.

(4) *Eurinoscopus*. The characteristics mentioned for *Eucselis* are still more marked in *Eurinoscopus*. Tissue damage is more severe and may occur in parenchyma (*M*). A number of tracks run both to phloem and xylem (*L*), but some tracks terminating in phloem are quite simple and apparently do not cause much tissue damage.

(5) and (6). Reference to the figures of *Erythroneura* and *Nesoclutha* feeding tracks will illustrate that these species cannot be effective vectors of phloem-restricted viruses.

(b) Differences in Feeding Tracks in Different Plant Organs, Plant Species, etc.

Feeding tracks of some of the same insects were studied in other plants and in leaves. Similarly the tracks of *Orosius* nymphs were studied to compare them with those produced by adults. The results are presented in Table 2.

These data when compared with those in Table 1 show that the feeding tracks of a single species terminate in the same tissues in varying frequencies depending upon the species of plant. Thus, *O. argentatus* tracks terminate in the phloem in 54 per cent. of cases on *Malva*, in 48 per cent. of cases on *Beta*, and in 2 per cent. of cases on *Datura*, when the petiole is almost too large to permit the stylets reaching the phloem. (The longest track left by *Orosius* was found in such a *Datura* petiole and measured about 500 μ in length.) Similarly, *Eurinoscopus* reached the phloem on 37 per cent. of occasions in *Malva* but on 50 per cent. of occasions on lucerne, in which the phloem occupies the major portion of the circumference of the stem at the depth to which the stylets of the insect can penetrate.

When feeding on the leaf the jassids will almost invariably reach the phloem if they feed on the under surface of the leaf veins. They do, in fact, appear to have a preference for such situations, as was indicated by the observations upon feeding on the *B. vulgaris* leaf (Table 2). The feeding tracks

of nymphal instars of *Orosius* produced tracks averaging only slightly shorter than those of the adults (respectively 230 μ and 270 μ) in large *Datura* petioles but otherwise were similar.

TABLE 2

TISSUES IN WHICH FEEDING TRACKS OF JASSIDS TERMINATE

			Percentage of Tracks in		
Jassid	Host Plant and Tissue	Total Tracks Counted	Phloem	Xylem	Paren- chyma or Mesen- chyma
O. argentatus adults	*Beta vulgaris* petiole	114	48	4	48
O. argentatus adults	Large *Datura stramonium* petiole	53	2	1	97
O. argentatus 1st-3rd instar	*B. vulgaris* petiole	112	23*		77
O. argentatus 4th instar	*B. vulgaris* petiole	106	18*		82
O. argentatus adults	*B. vulgaris* leaf	55	29	2	69
E. punctatus adults	*Medicago sativa* stems	54	50	24	26

* Most of these tracks terminated in the very small bundles in the "wings" of these petioles, which were unusually large, and in these bundles it was impossible to determine precisely where the insect was feeding.

(c) Ability of the Jassids to Distinguish the Tissues of Plants

The following is a summary of observations that together suggest strongly that some jassids can distinguish tissues of plants upon which they feed. (1) The feeding tracks of *Orosius* are frequently larger when they terminate in the phloem than when they terminate in the xylem. (2) *Limotettix* certainly feeds more frequently on phloem than on other tissues. (3) The frequency of tracks curving towards the phloem is greater than the frequency of those curving away from it (Figs. 1-6). (4) Observations on the behaviour of *Limotettix, Orosius,* and *Euscelis* show that a preponderance of the insects feed on the veins on the ventral surface of leaves in preference to other parts of the plant.

The conclusion seems justified that some species of jassids show a preference for feeding on the phloem tissue.

III. AN INVESTIGATION OF THE pH GRADIENT HYPOTHESIS

If, as has been shown in the previous section, some jassids show a preference for feeding on phloem tissues, the next question concerns the method by which they recognize or locate this tissue. Fife and Frampton (1936)

suggested the attractive hypothesis that *Circulifer* (*Eutettix*) *tenellus* finds the phloem of the sugar beet by a pH gradient from the parenchyma towards the phloem. It seemed possible that differences in infectivity rates in crops attacked by *Orosius argentatus* might be explained by the varying ability of this jassid to locate the tissues in each crop in which the virus could multiply, and it was therefore decided to repeat Fife and Frampton's experiment on several host plants of *Orosius*. Gradients of pH from the epidermis to the phloem were sometimes found in some of these plants, but not in others. The gradients when present were generally much less marked than those reported by Fife and Frampton. The evidence for these conclusions is given in the remainder of this section.

TABLE 3

pH MEASUREMENTS IN PETIOLES OF SUGAR BEET PLANTS GROWN UNDER SIMILAR GREENHOUSE CONDITIONS. TWO PETIOLES FROM EACH PLANT

Tissue	Plant 1		Plant 2		Plant 3		Plant 4		Plant 5	
Parenchyma	5.6	5.9	5.5	5.6	5.8	5.8	5.7	5.9	6.0	6.1
Parenchyma	5.6	5.8	5.6	5.7	—	—	—	—	—	—
Mech. tissue	5.7	5.7	5.9	5.7	—	—	—	—	—	—
Phloem	5.7	5.8	5.8	5.7	6.1	6.1	6.2	6.2	6.8	6.3
Xylem	5.9	5.9	5.6	5.9	—	—	—	—	—	—

(*a*) *Methods*

The method described by Fife and Frampton employs a modified quinhydrone electrode and is open to a number of objections. The quinhydrone method gives low readings and is not accurate above pH 8.0. It is liable to protein errors and is not suitable in the presence of oxidizing or reducing agents. In addition, the micro-scale modification suffers from uncertainties due to quinhydrone solubility in cell sap, tissue damage in the phloem even by the 40 μ diameter electrodes, and the effect of the saturated KCl on the plant tissues. Nevertheless, the method was found to be the most suitable of any used and an attempt has been made to follow the technique of Fife and Frampton as closely as possible. Platinum needles were soldered to short, shielded leads and sealed in glass tubes. They were tested on a series of buffers from pH 4.0 to 8.0, saturated with quinhydrone. Only electrodes that responded over this range with an accuracy of ± 0.1 pH unit were used. The Cambridge bench-type pH meter used was standardized before and after each series of tests on a buffer solution at pH 4.0 (0.05M phthalate) and checked on a buffer solution of pH 8.0 (0.067M phosphate). Special precautions were taken with leads, junctions, earthing, shielding, bridges, cleaning of electrodes, and similar details.

Much the same results were obtained when the electrical connection with the standard calomel electrode was made through the length of the petiole, as in Fife and Frampton's work (in which the resistance between the electrodes approximated 100,000 ohms), or when this connection was achieved by means

of an asbestos fibre sealed in a glass capillary filled with saturated KCl solution and immersed in the cell sap on the cut surface of the petiole a few cells from the platinum electrode (Fig. 7). Resistance between the electrodes using this method approximated 70,000 ohms. The pH meter was capable of measuring potentials in systems with resistances of 100,000,000 ohms.
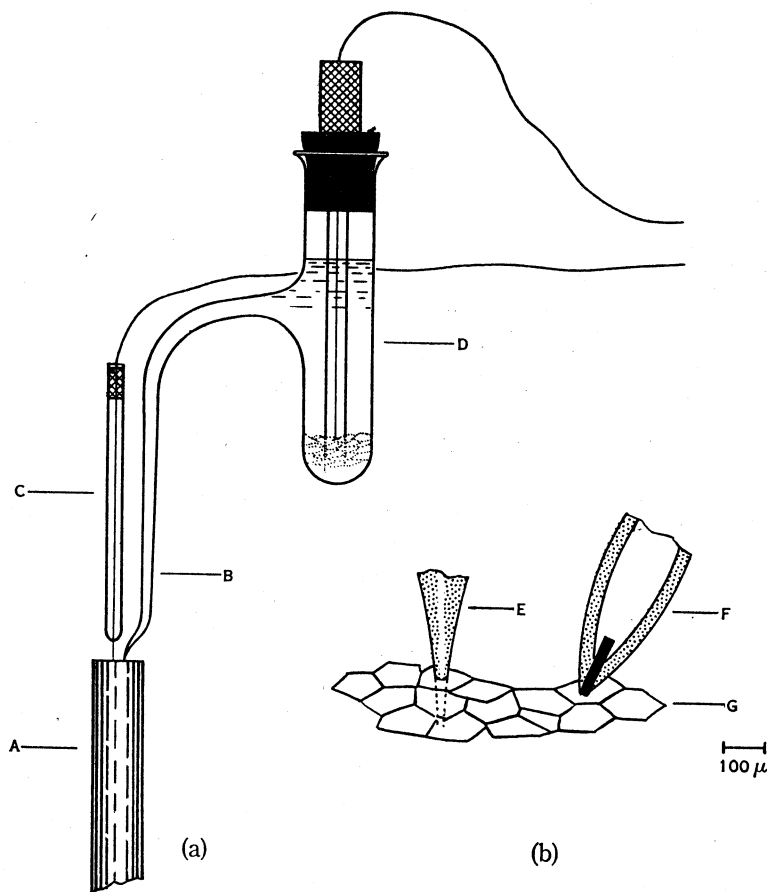

Fig. 7.—Diagram of electrode assembly for measuring pH of plant cell sap.
(a) Calomel half cell and platinum electrode applied to the cut surface of a petiole.
(b) Detail of immersion of electrodes in cell sap.
A, petiole; B, KCl bridge with asbestos fibre sealed in a capillary tip;
C, platinum electrode; D, calomel half cell; E, platinum needle; F, tip of KCl bridge with asbestos fibre; G, cut surface of a petiole.

(b) Results

A number of readings with this apparatus have been made. In Tables 3-6 inclusive the vertical columns represent a series of pH readings progressing from the epidermis towards the vascular bundles. Table 3 gives the pH

TABLE 4

pH MEASUREMENTS IN PETIOLES OF SUGAR BEET PLANTS GROWN UNDER DIFFERENT CONDITIONS

A, greenhouse; B, outdoors

Tissue	A1		A2		A3		A4		B1		B2		B3		B4	
Parenchyma	5.6	5.4	—	—	—	5.9	—	6.1	6.0	6.1	6.0	5.6	5.9	6.0	5.6	5.7
Parenchyma	5.9	5.6	5.9	5.6	5.9	—	—	6.1	5.8	6.0	6.1	5.9	6.3	6.5	5.7	5.9
Parenchyma	5.7	5.6	5.8	5.9	—	—	5.9	6.1	6.0	6.2	6.3	6.2	6.5	6.8	6.0	6.1
Mech. tissue	5.7	5.7	5.7	5.8	6.2	—	6.1	6.0	6.2	6.5	6.4	5.9	7.3	7.3	6.2	6.3
Phloem	5.8	5.7	5.7	5.9	—	—	6.0	6.2	6.5	6.3	6.7	6.3	7.8	7.3	6.5	6.2
Xylem	5.8	5.9	5.8	6.0	—	—	6.1	5.9	6.1	5.9	6.6	6.1	7.2	7.0	5.8	5.8

TABLE 5

pH MEASUREMENTS IN PETIOLES OF FOUR SUGAR BEET PLANTS

A—a normal plant from greenhouse
B—the same plant from greenhouse; petioles upon which *Nezara* had fed
C—virus (Big Bud) infected plant from greenhouse
D—infected plant from outdoors

Tissue	A		B		C	D					
	16.ii.51	21.ii.51	21.ii.51	21.ii.51	19.ii.51	14.ii.51	14.ii.51	15.ii.51	15.ii.51	19.ii.51	19.ii.51
Parenchyma	5.4	5.6	5.8	5.6	5.6	6.0	5.9	6.3	6.3	5.6	5.7
Parenchyma	5.6	5.8	5.8	5.7	5.9	6.5	6.3	6.1	6.0	—	—
Parenchyma	5.6	—	—	—	—	6.8	6.5	6.2	6.2	—	—
Mech. tissue	6.7	5.8	5.8	5.8	5.9	7.3	7.3	6.7	6.4	—	—
Phloem	5.7	5.8	5.7	6.0	6.0	7.3	7.8	7.4	7.1	6.5	6.6
Xylem	5.9	5.8	5.7	5.9	5.7	7.0	7.2	5.7	5.5	—	—

measurements on a series of sugar beet petioles grown under practically identical conditions in the greenhouse. They indicate a plant to plant variation in pH of the different tissues (greatest range in phloem pH 5.7 to 6.8); and even between two petioles from the same plant substantial differences in pH exist (greatest range in phloem pH 6.3 to 6.8). More important, it will be noted that in this series the maximum difference (in plant No. 5) between parenchyma and phloem is about 0.8 pH units. In most the difference is much smaller than this, and the same applies to the bulk of the data presented in this section, whereas Fife and Frampton report a range of 1.6 pH units or more.

Table 4 presents similar data for two series of plants, the first grown in the greenhouse and the second grown outdoors. The conclusion seems justified that the results in the second series are higher than those in the first, but in neither is there always a progressive pH gradient from parenchyma to phloem, although in some of the plants grown outdoors such a gradient undoubtedly occurs. On the contrary in plant A_4 the pH of the parenchyma is almost the same as that in the phloem.

In Table 5 data are presented which indicate that the pH of petioles from the same plants may change from day to day. The data (column B) also suggest that the feeding of certain Hemiptera on a petiole does not in itself result in pH changes and that infection by the virus of Big Bud of tomato in the beets did not cause marked changes in pH (compare column D with plants B_{1-4} in Table 4).

TABLE 6

pH MEASUREMENTS IN PETIOLES FROM DIFFERENT SPECIES OF PLANTS

Tissue	Pumpkin		Tomato		Tobacco	
Parenchyma	5.9	5.9	5.7	6.1	5.5	5.4
Parenchyma	5.8	5.9	5.9	6.1	5.5	5.4
Parenchyma	6.1	5.8	6.1	6.1	5.5	5.4
Phloem	6.4	6.4	6.2	6.2	5.9	6.0
Xylem	6.4	6.3	6.2	6.2	5.8	6.3

Table 6 gives representative data showing that the conditions in sugar beet are found also in other species. In some petioles pH gradients were observed, in others, no such gradients. In pumpkins it was regularly observed that the tissues of the vascular bundles were less acid than the parenchymatous tissue. However, the pH of the phloem and of the xylem were not greatly different in most petioles in cucurbits.

(c) Critique of the Hypothesis of pH Gradient as a Factor in Assisting Jassids in Finding the Phloem

A consideration of the following points suggests that no gradient of any kind assists the jassids studied in locating the tissues on which they prefer to feed.

(1) Observations on the feeding tracks referred to in Section II(*b*) do not suggest that the insects were responding to a gradient. The number of straight tracks greatly exceeds the number that curve in the species studied.

(2) *Orosius* adults were fed on young leaves of pumpkin. Feeding tracks did not terminate in the vascular bundles with any greater frequency than in plants that did not have alkaline vascular bundles. Similarly, *Orosius* were fed on *Beta* plants differing in the pH of their tissues (plants B_1 and B_3 of Table 4). No difference was found in the distribution of feeding tracks in these two plants.

(3) The data of Day and McKinnon (1951, Table 8) on the amount of material ingested of pH from 4.2 to 9.0 lends no support to the theory that *Orosius* reacts to the alkalinity of its food.

(4) Examination of the data presented by Fife and Frampton (1936, Table 3) and our Tables 1 and 7 suggests that the total number of insects reaching the phloem in their work was not much greater than those in our experiments. Admittedly it is not easy to make a direct comparison because of differences in the methods of presenting the data, but with *Circulifer* on sugar beet, about 55.8 per cent. reached the phloem compared with *Orosius* in which 48 per cent. of the feeding tracks terminated in the phloem.

IV. How do the Jassids Find the Tissues upon which They Prefer to Feed?

In the previous section evidence has been presented that certain jassids at least do not utilize a pH gradient to find the phloem; no alternative hypothesis has previously been suggested. Data substantiating such an alternative will now be given.

Examination of Table 1 and Figures 1-6 indicates that the species studied fall into three groups:

(*a*) The species of *Erythroneura* and *Nesoclutha* are evidently primarily parenchyma feeders, as were the majority of species of *Empoasca* studied by Smith and Poos (1931), and the jassids described by Smith (1926).

(*b*) In the second group of jassids are included species whose feeding tracks reach the phloem in about 30-50 per cent. of cases, i.e. the species of *Orosius, Eurinoscopus*, and *Euscelis*. These can distinguish the phloem when they reach it, but apparently feed also on the parenchyma. The principal evidence for the former statement comes from the observation that the tracks that terminate in the phloem are usually considerably larger than those in the parenchyma. Evidence suggesting that these species feed on the parenchyma comes from two observations: (i) *Orosius* have been shown by Day and McKinnon (1951) to feed on a variety of liquids, including distilled water (containing tracer quantities of ^{32}P); (ii) they live for some days when provided only with delaminated stems of *Datura* in which they are unable to reach the vascular bundles.

Measurements of the relative proportions of the circumference occupied by phloem and parenchyma at a depth to which the stylets can penetrate in

Malva and *Beta* petioles are given in Table 7. Comparison of these data with those in the last column of the table (taken from Tables 1 and 2) indicates that the percentage of feeding tracks terminating in the phloem is of the order of magnitude of the relative sections of phloem and parenchyma. Only approximate figures can be given because of the variability in different regions of a single petiole but the figures are reasonably accurate.

There is good evidence, therefore, that the species studied belonging to the genera *Orosius, Eurinoscopus,* and *Euscelis* feed on whatever tissues they reach with preliminary probing, but that they feed longer on the phloem than on the parenchyma.

(c) The third type of feeding behaviour is exemplified by *Limotettix*. In this, the insect clearly reaches the phloem a significantly greater number of times than it would by chance. From Figure 1 it will be observed that the number of curving feeding tracks is too low in *Limotettix* for them to account for the high percentage of tracks terminating in the phloem. This in itself is evidence that this species does not employ a gradient of any sort to assist in finding the phloem.

TABLE 7

COMPARISON BETWEEN APPROXIMATE SECTION OF PLANT TISSUES AND NUMBER OF FEEDING TRACKS TERMINATING IN PHLOEM

Species of Plant	Approximate Mean Percentage Cross Section of		Percentage of Feeding Tracks of *Orosius* Terminating in Phloem
	Parenchyma	Phloem	
Beta	50	50	48
Malva	40	60	54

Examination of Figure 1 (especially the tracks at *C*) suggests that *Limotettix* must either detect the vascular bundle from the exterior of the petiole or that, in probing, it generally leaves no feeding tracks unless it reaches the phloem. The first hypothesis is difficult to accept but direct evidence for the second results from a comparison of measurements made of the time *Orosius* and *Limotettix* spend in feeding on the petioles of *Malva*. It was found that 36 per cent. of the *Orosius* had feeds of less than 4 minutes duration whereas 80 per cent. of the *Limotettix* fed for less than 4 minutes. It was obvious that *Limotettix* was more restless than *Orosius*, and its normal behaviour when alighting on a *Malva* petiole is for it to probe two or three times before settling to feed, whereas *Orosius* normally feeds the first time it probes the plant tissues. It seems very likely that *Limotettix* leaves no feeding tracks in its preliminary probings.

These data suggest that the tissues reached by the stylets of *Limotettix* depend solely upon chance, and thus bring this species into line with those considered in the first two groups.

Fife and Frampton (1936) present some data (their Table 3) that require an explanation, namely the reduction in the number of feeding tracks of *Circulifer* that terminated in the phloem after CO_2 treatment. If the mean percentage cross section of phloem approached that of the petioles used in the present investigation it is difficult to understand how only 12.12 per cent. of feeding tracks could have terminated in the phloem unless the insects were actually repelled by that tissue. The greatest increase in the number of feeding tracks in the CO_2-treated plants is in the group in which the punctures "were not in direction to hit phloem and not deep enough." It is possible that the treatment made the tissues unpalatable, but in view of the report that the amount ingested by Orosius was not greatly influenced by pH (Day and McKinnon 1951), this explanation does not seem likely. Further work on this problem raised by Fife and Frampton's observation is clearly needed.

V. Movements of the Mouth-Parts, Stylet Sheath Formation, etc.

The penetration of plant tissues by the mouth-parts cannot be observed directly, but there is no reason to believe that the mechanism differs from that which can be observed when the insect is induced to puncture a transparent plastic membrane as described by Day and McKinnon (1951). The insect braces its prothoracic and mesothoracic legs, rotates the head so as to bring the rostrum (which normally is directed posteriorly between the prothoracic legs) perpendicular to the surface to be penetrated. The surface of a plastic membrane is actually dented by the pressure of the mouth-parts and almost immediately the feeding stylets, comprising the mandibles and maxillae, appear through the membrane, and extend about 70 μ into the fluid. The insect may then either withdraw the stylets and move off, leaving no trace of its presence, or settle to feed. In the latter event it proceeds to form a salivary sheath. The stylets perform a fairly rapid oscillating motion. As they are withdrawn a small particle appears at the tip. This is shaped into a tube-like sheath by the next forward movement. Each movement adds to the length of the sheath at its distal end (although sometimes the sheath forks at the tip). It reaches a length of about 70 μ in a minute or so, but may be added to at any time subsequently during feeding. The stylets then extend beyond the sheath (sometimes for brief periods to more than three times its length) and are moved to and fro in the fluid. This probably occurs during actual ingestion. Withdrawal of the mouth-parts from the sheath occurs after feeding is complete or if the insect is disturbed. It may be effected with considerable speed and without difficulty. The hind legs are placed on the membrane and the insect withdraws the stylets and moves away. If the disturbance is caused by another insect or change of light intensity, the stylets may be reinserted in a position close to the previous one, often without any other movement of the insect.

No important differences have been observed between any of the species studied; except that *Nesoclutha* and *Erythroneura* insert their mouth-parts at an

angle of about 45° with the membrane, whereas those of the other species usually do so perpendicular to it. The above description also agrees with that given by Storey (1939) for *Cicadulina mbila* feeding through a paraffin membrane, and we differ from Storey only with reference to the occurrence of material other than the salivary sheath flowing from the stylets. The observations were not conclusive on this point but we have visual evidence of the existence of such a substance.

The above observations give support to the conclusion that the insects feed at the termination of the feeding tracks as determined by Millon's reagent. The tracks are longer in plant tissues than they are in a fluid medium but there is no need in the fluid for longer tracks although, as mentioned above, occasional exploratory extensions of the stylets may occur.

VI. DISCUSSION AND CONCLUSIONS

It has often been observed by workers on jassid transmission of plant viruses that a jassid infects only some of a series of plants to which it is exposed. Bawden (1950, p. 87) considers it probable that such results indicate "that the salivary glands are easily exhausted of virus and that there is not a steady flow of virus from the blood to the glands, but that it takes place irregularly." It is difficult to conceive of a physiological mechanism that would account for varying rates of flow of the virus into the salivary glands. The results could, however, be explained on the basis of observations reported above, namely that the jassid may not reach the viruliferous tissue or the tissue in which the virus can develop every time it feeds. It seems probable also that certain of the results of Storey (1938) can be explained in the same way.

For a jassid to be an efficient vector of a phloem-restricted virus at least two conditions must be fulfilled with respect to its feeding behaviour. Firstly, it must reach the phloem in a reasonably high percentage of feeds, and secondly it must presumably not cause undue damage in the phloem, which might inhibit the development of the virus. The data presented in the previous sections permit an evaluation of the species studied as virus vectors on the basis of these two criteria. It should be emphasized that factors other than feeding behaviour, such as the permeability of the gut and salivary gland to the virus, may play a significant role in determining whether a species acts as a vector. The work of Storey (1933) illustrates this with the strains of *Cicadulina mbila*. However, it is equally true that the role of the feeding behaviour has been neglected in previous work. One further qualification should be appreciated. The data of Table 1 were obtained from insects feeding on the petioles of *Malva*. Although it is suggested from other data, such as some of that in Table 2, that these figures give a guide to the feeding behaviour, it is true that a jassid will reach the phloem of many plants whenever it feeds on the underside of a small leaf vein. The opportunity for transmission of plant viruses by jassids is, therefore, greater than is indicated by the crude

numerical values in Table 1, especially when the exposure times are sufficient for several feeding periods. Our results indicate that the species studied make approximately six feeding tracks per day at room temperatures.

With these qualifications it will be clear that the ability of the insects studied to act as vectors of phloem viruses, on the basis of feeding behaviour alone, is in the following order: *Limotettix, Orosius, Euscelis, Eurinoscopus*. Neither *Erythroneura* nor *Nesoclutha* would be expected to act as vectors of phloem-restricted viruses.

Limotettix would appear to be the most efficient vector, both because of its clean feeding tracks, which cause very little tissue disturbance, and because of its ability to find the phloem in a high percentage of feeds. *Orosius* has clean feeding tracks but is significantly less efficient in finding the phloem. *Euscelis* caused more tissue damage, whereas *Eurinoscopus* caused quite severe necrosis at the sites of feeding. The amount of injury seems to increase with the size of the jassid. It is noteworthy that vectors of plant viruses are mostly small jassids. However, it is conceivable that a certain amount of tissue damage might provide an accumulation of protein (Smith 1933), which could be a suitable medium for the establishment of a virus infection. Tests are planned to determine the ability of the jassids mentioned above to act as vectors of certain plant viruses.

VII. Acknowledgments

VIII. References

BAWDEN, F. C. (1950).—"Plant Viruses and Virus Diseases." 3rd Ed. (Chronica Botanica Co.: Waltham, Mass.)

BENNETT, C. W. (1935).—Studies of properties of the curly top virus. *J. Agric. Res.* **50**(3): 211-41.

BENNETT, C. W., and WALLACE, H. E. (1938).—Relation of the curly top virus to the vector, *Eutettix tenellus. J. Agric. Res.* **56**: 31-51.

DAY, M. F., and McKINNON, ANNE (1951).—A study of some aspects of the feeding of the jassid *Orosius*. *Aust. J. Sci. Res.* B 4(1): 125-35.

FIFE, J. M., and FRAMPTON, V. L. (1936).—The pH gradient extending from the phloem into the parenchyma of the sugar beet and its relation to the feeding behaviour of *Eutettix tenellus*. *J. Agric. Res.* 53(8): 581-93.

HOUSTON, B. R., ESAU, K., and HEWITT, W. B. (1947).—The mode of vector feeding and the tissues involved in the transmission of Pierce's disease virus in grape and alfalfa. *Phytopathology* 37: 247-53.

SMITH, F. F. (1933).—The nature of the sheath material in the feeding punctures produced by the potato leafhopper and the three-cornered alfalfa hopper. *J. Agric. Res.* 47: 475-85.

SMITH, F. F., and POOS, F. W. (1931).—The feeding habits of some leaf hoppers of the genus *Empoasca*. *J. Agric. Res.* 43(3): 267-85.

SMITH, K. M. (1926).—A comparative study of the feeding methods of certain Hemiptera and of the resulting effects upon the plant tissue, with special reference to the potato plant. *Ann. Appl. Biol.* 13: 109-39.

STOREY, H. H. (1933).—Investigations of the mechanism of the transmission of plant viruses by insect vectors. I. *Proc. Roy. Soc.* B 113: 463-85.

STOREY, H. H. (1938).—Investigations of the mechanism of the transmission of plant viruses by insect vectors. II. The part played by puncture in transmission. *Proc. Roy. Soc.* B 125: 455-77.

STOREY, H. H. (1939).—Investigations of the mechanism of the transmission of plant viruses by insect vectors. III. The insect's saliva. *Proc. Roy. Soc.* B 127: 526-43.