THE FEEDING SITES IN *SONCHUS OLERACEUS* OF *HYPEROMYZUS LACTUCAE*, THE APHID VECTOR OF LETTUCE NECROTIC YELLOWS VIRUS

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Summary

The feeding sites of the aphid *H. lactucae* on *S. oleraceus*, the natural wild plant host of lettuce necrotic yellows virus, have been investigated. Of 159 identifiable terminal feeding sites seen in transverse sections of *S. oleraceus* stem, 92·5% ended in the phloem and most of these were in the relatively young cells, 6·9% terminated in parenchyma cells, and 0·6% in xylem. The high proportion of sites in the phloem indicates that lettuce necrotic yellows virus is probably acquired from that tissue.

I. Introduction

Lettuce necrotic yellows virus (LNYV) was first described by Stubbs and Grogan (1963), who showed it to be transmitted by the thistle aphid [*Hyperomyzus lactucae* (L.)] from sowthistle (*Sonchus oleraceus* L.) to lettuce (*Lactuca sativa* L.). Infected *S. oleraceus* exhibits no obvious symptoms but *L. sativa* is severely affected by this virus. LNYV has been observed in host leaf tissue (Chambers, Crowley, and Francki 1965), in xylem vascular tissue (Chambers and Francki 1966), and more recently it has been established that the virus becomes systemic through the tissues of the aphid vector *H. lactucae* (O'Loughlin and Chambers 1967).

The complete virus particle is large and unusually complex in structure. It is bacilliform in shape (approximately 66 by 227 mμ) and apparently membrane-bounded (Wolanski, Francki, and Chambers 1967). Although up to the present time the particles have been seen with certainty only in mesophyll tissues of the leaf and in young xylem vessels in vascular tissue of leaf and petiole, the pattern of infection of a plant is strongly suggestive of a phloem-translocated virus. It was considered that a study of the locations of aphid feeding sites in *S. oleraceus* might indicate the tissues from which LNYV is normally acquired by the vector.

In a comprehensive review of aphid feeding and nutrition Auclair (1963) summarized histological studies of the feeding tracks of some 46 aphid species. It seems clear from this review that phloem is the principal feeding site of most species and it seems generally agreed that the sieve tubes are the cells tapped by the stylets of the phloem feeders. The data for some species suggests that they may feed in more

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than one cell type. For example, Davidson (1923) studied the feeding sites of *Aphis rumicis* and found that on beans the phloem was the chief tissue tapped, but that cortex and mesophyll cells of the leaf may also be penetrated, while in *Rumex*, the xylem was frequently tapped for food. Diehl and Chatters (1956) reported that the spotted alfalfa aphid *Therioaphis maculata* when feeding on alfalfa appeared to make many more stylet terminations in the parenchyma cells than in the phloem.

The duration of feeding by the aphid determines the type of tissue reached. Esau, Namba, and Rasa (1961) studied the penetration of sugar-beet leaves by the aphid *Myzus persicae* by observation of the salivary sheaths remaining in the tissues after withdrawal of the stylets. They found that after naturally terminated penetrations of short duration (i.e. less than 30 min), the phloem was rarely reached, but that after access periods of "longer duration", most stylets reached the phloem. These authors suggested that the short intercellular stylet tracks were probably the result of probing without feeding, but they could not offer an explanation for the high percentage of deep penetrations which did not end in the phloem cells. Skotland and Hagedorn (1955) had also found that the depth of stylet penetration depended on the feeding time and that an hour or so may be required for the phloem to be reached.

In an attempt to ascertain from which cells LNYV is acquired by the aphid, this study reports on the feeding sites in *S. oleraceus* of *H. lactucae*, the only known vector of this virus.

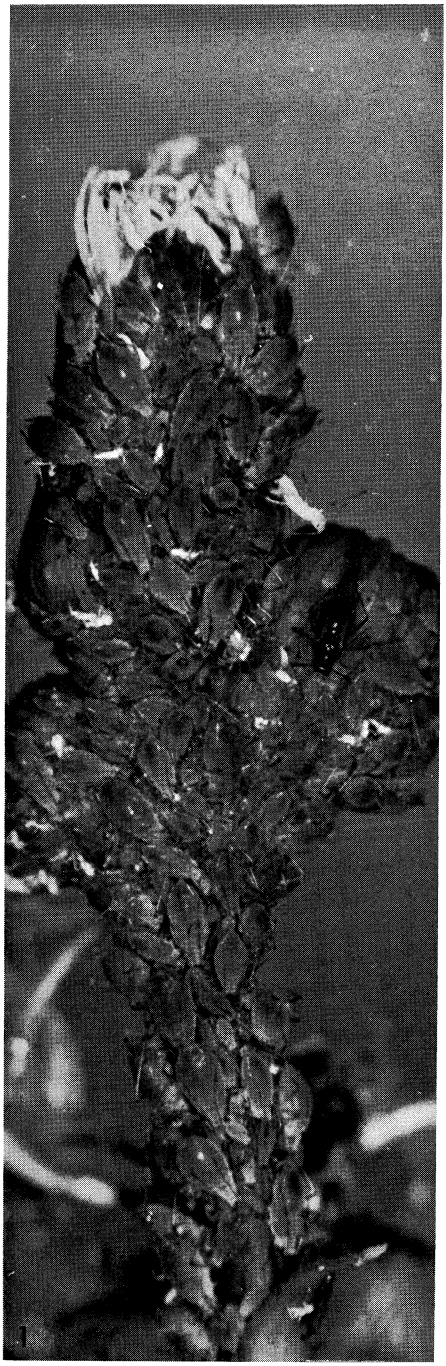
II. Materials and Methods

In the present study we were primarily interested in the normal feeding site and therefore used naturally infested plants on which the aphids had become well established (Fig. 1). Several methods (Dykstra and Whitaker 1938; Ledbetter and Flemion 1954; Diehl and Chatters 1956) have been described for obtaining sections of plant containing the stylets of sucking insects in their normal feeding positions. To achieve this it is necessary to kill the insect or sever the stylets without disturbing them.

The effect of different killing fluids on stylet retraction in *H. lactucae* was tested by removing feeding aphids and placing them immediately into the fixing fluid while the stylets were still extended. In Carnoy's fixative the aphids continued to move for up to 30 sec. In chloroform all movement ceased within 2 sec. In these fluids the stylets were not noticeably retracted.

Formalin–acetic acid–60% ethanol (10 : 5 : 85 v/v) was found to be a suitable fixative both for the *S. oleraceus* and the whole aphid tissues. The procedure finally used for fixation of *H. lactucae* while on *S. oleraceus* was a modification of the method used by Dykstra and Whitaker. On a cold morning, short lengths of *S. oleraceus* stem on which aphids were feeding were carefully removed from a naturally infested plant (a typical such heavily infested shoot is illustrated in Fig. 1). The stem pieces were placed in a refrigerator at 4°C for about 5 min which immobilized the aphids. They were then placed in chloroform for 15 sec and transferred to formalin–acetic–ethanol fixative, where they remained 2–20 days before being dehydrated and wax-embedded. The procedures used were standard and included dehydration using a t-butanol–ethanol series (Johansen 1940) followed by infiltration with 59°C paraffin and serial transverse sectioning.

As safranin O proved an excellent stain for the salivary sheaths which remain after the stylets have been withdrawn from plant tissue, it was included in all the stain combinations tested. The most satisfactory staining method was found to be iron-mordanted safranin O and celestine blue (Gray and Pickle 1956) followed by fast green and orange G (Johansen 1940). It was found that fairly thick sections (15 μ) were most satisfactory as entire stylet tracks were frequently contained within the one section.



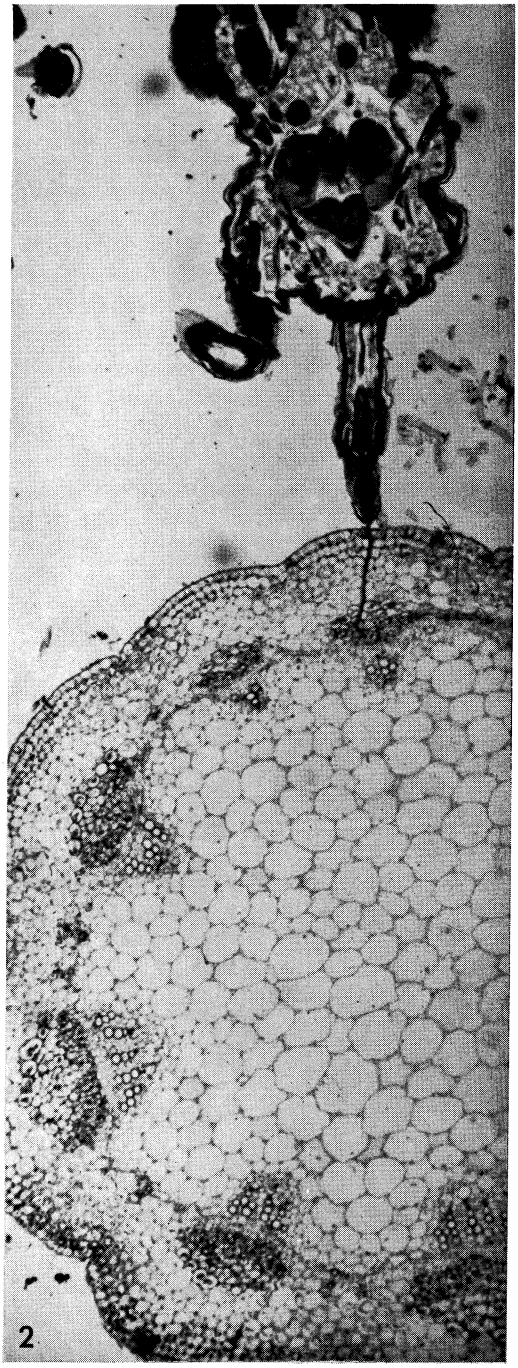


Fig. 1.—A heavy infestation of *H. lactucae* on an *S. oleraceus* shoot. ×4·5.
Fig. 2.—A section of *S. oleraceus* and of *H. lactucae* showing the aphid stylets inserted into phloem tissue. ×100.

III. Results

Of the 1,185 sections of aphid-infested *S. oleraceus* examined, only one was seen which contained the whole length of the sectioned proboscis plus the stylets in their feeding position (Figs. 2 and 4). Stylet tracks were recognized either by the stylets which they contained or, as in the majority of cases, by the stained salivary sheaths which remained after the aphids had withdrawn their stylets (Fig. 3).

Although often difficult to judge, it appeared that these tracks and the stained salivary sheaths were mainly intercellular. High-magnification light photomicrographs indicated that the tips of the maxillary stylets are separated when they are intracellular and this presumably is their normal position while the aphid is feeding. This is illustrated in the phase-contrast light micrographs of Figures 6 and 7. Stylets such as those illustrated in Figures 5 and 6 have evidently partly withdrawn and altered their positions as indicated by the stained salivary sheaths remaining in the earlier sites (see arrows). The vascular bundles were frequently ill-defined in the outer phloem area. Cell counts sometimes included parenchyma which may not have been phloem.

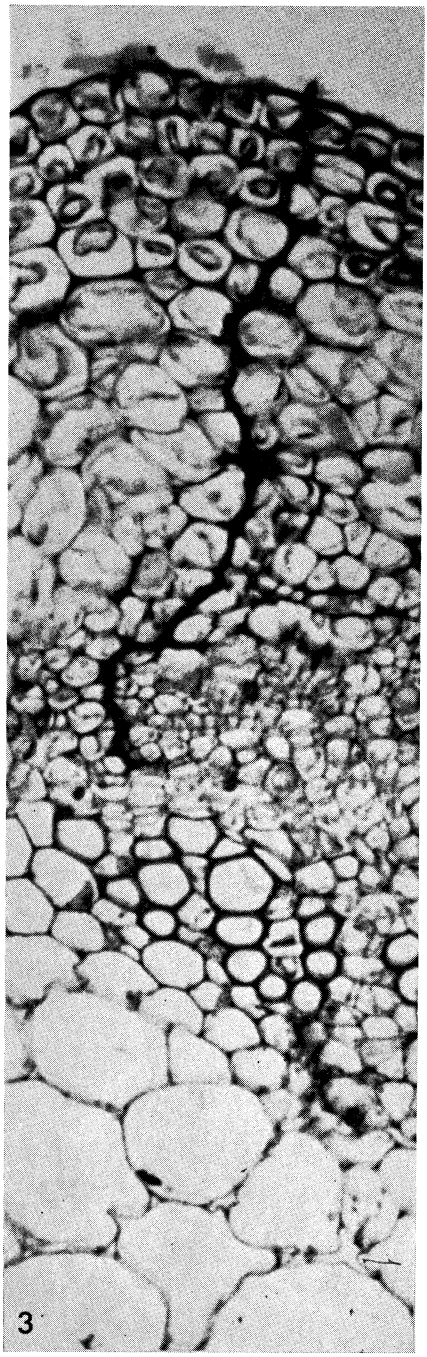
Of the many tracks examined, 159 were sufficiently well preserved to enable terminal sites to be identified; 92·5% terminated in the phloem—this included 18 tracks which still contained the stylets, and 129 which consisted of salivary sheaths; 6·9% terminated in parenchyma cells (a total of 11 salivary tracks); while only 0·6% (i.e. one salivary track) appeared to terminate in the xylem.

The terminal positions of 124 tracks within the phloem could be judged accurately in relation to the distance from the cambium. These are plotted in Figure 8 which is a diagrammatic representation of the radial rows of phloem cells in vascular bundles of various sizes within young *S. oleraceus* stems. Each dot indicates the position of one feeding site in relation to the fascicular cambium, whilst the height of each column represents the depth of phloem. The paucity of terminal sites in the larger bundles is possibly misleading. This is not to be interpreted as meaning that few penetrations terminate in such large bundles, but rather reflects the relatively few bundles (11%) with phloem 12–15 cells wide found in the young stems investigated. The diagram does illustrate, however, that the feeding sites are fairly randomly distributed through the inner and presumably functional phloem and a number are in the youngest secondary phloem cells immediately adjacent to the cambium.

IV. Discussion

The high proportion of tracks terminating in the phloem clearly shows that *H. lactucae* is primarily a phloem feeder when on *S. oleraceus*. This means that LNYV, which systemically infects *H. lactucae* (O'Loughlin and Chambers 1967), is almost certainly introduced into and collected from *S. oleraceus* via the phloem tissues.

Unfortunately it has not been possible to study the stylet tracks in lettuce plants. The aphid does not breed on lettuce and is a reluctant feeder on this species, even though it is the proven and apparently only vector of the virus to lettuce plants (Stubbs and Grogan 1963). However, McLean and Kinsey (1968) have shown that



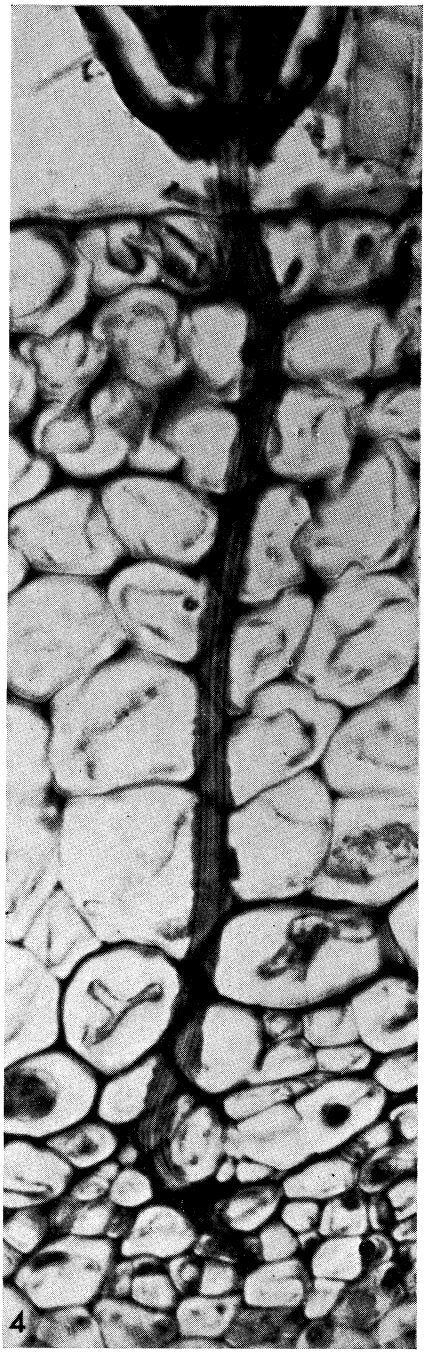


Fig. 3.—A section of *S. oleraceus* in which a salivary sheath terminating in the phloem remains after withdrawal of the aphid stylets. ×450.

Fig. 4.—A higher magnification of Figure 2 showing details of the aphid stylets in their feeding position. ×900.

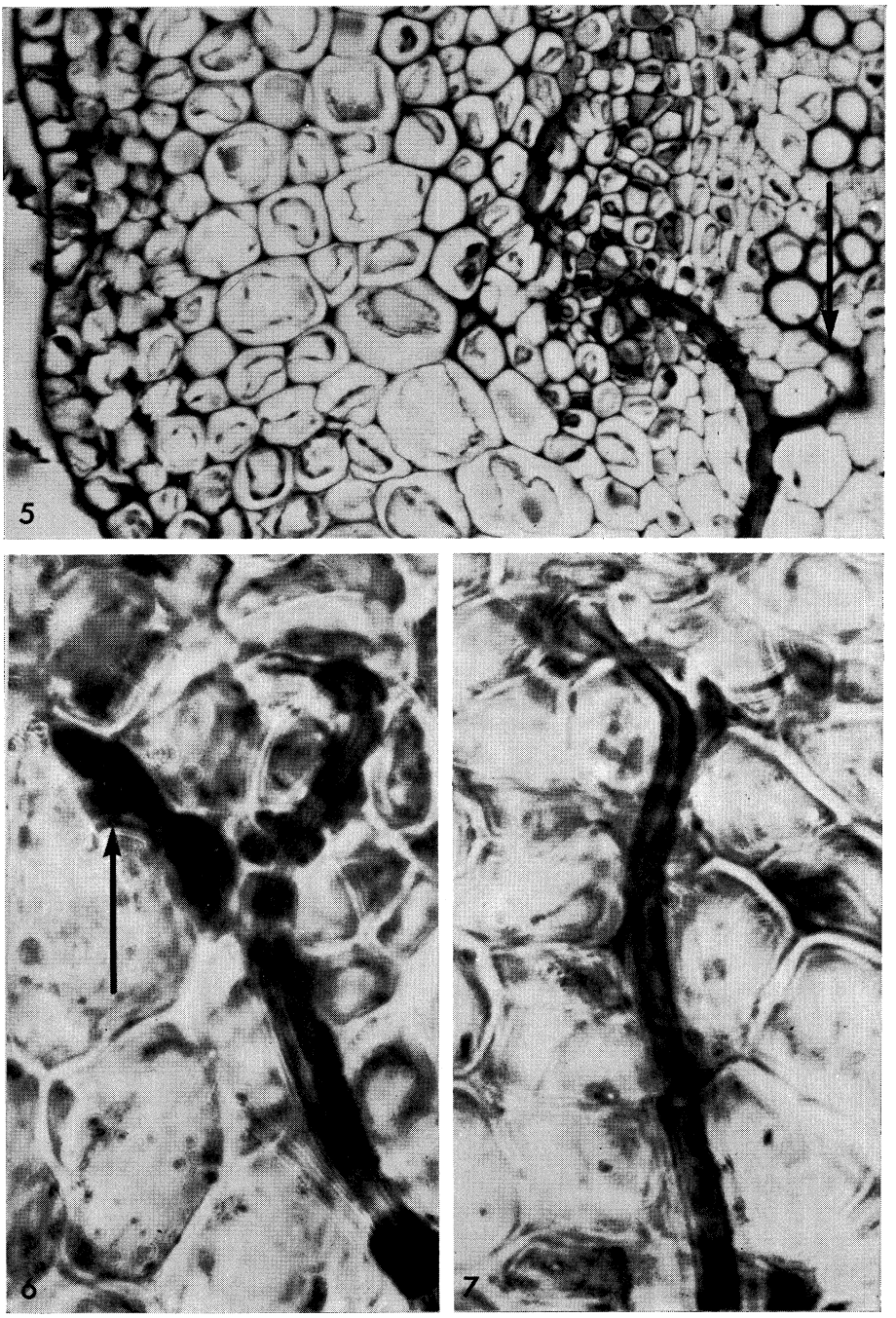


Fig. 5.—In this section of *S. oleraceus* a salivary sheath penetrating a xylem tracheid (see arrow) indicates that this was not a suitable feeding site as the stylets now terminate in phloem tissue. $\times 560$.

the aphid *Acyrthosiphon pisum*, when probing lettuce (a non-host plant), ingests sap from epidermal, subepidermal, and mesophyll parenchyma cells. Aphids whose stylets reached the phloem rarely ingested sieve tube sap. This might apply also to *H. lactucae* on the non-host lettuce, in which case LNYV would be injected into cells other than those of the phloem.

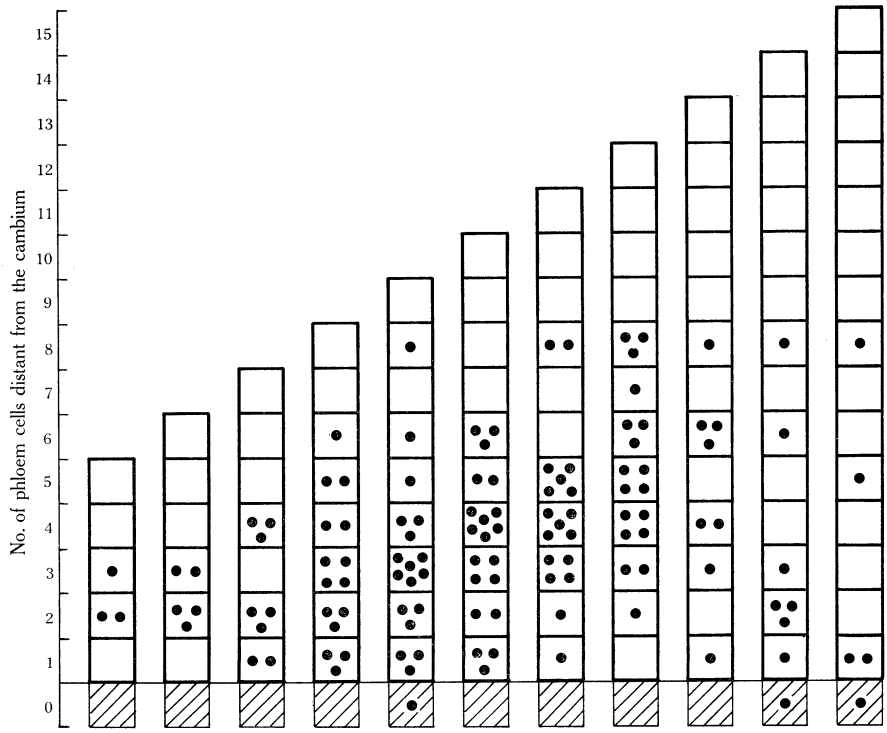


Fig. 8.—Representation of aphid feeding sites in phloem tissue in relation to their distance from the cambium (cross-hatched squares) in vascular bundles of different sizes. Each column represents a phloem group and shows the number of phloem cells counted radially through the thickest part of each vascular bundle. Each dot marks a terminal feeding site and its position indicates the number of cells distant from the cambium.

We have some data further supporting our view that the vector feeds from sieve-tube elements in *S. oleraceus*. This is indicated by the presence in the vector mid-gut lumen of material similar to that described by Cronshaw and Esau (1967) as occurring in sieve-tube elements of *Nicotiana tabacum*.

There is a considerable amount of data available on the feeding habits of other species of aphids. Watson and Nixon (1953), by feeding *Myzus persicae* on radioactive

Fig. 6.—Phase-contrast micrograph illustrating the separated curved tips of the maxillary stylets. A previous position is indicated by the darkly stained salivary sheath (see arrow). ×1890.
Fig. 7.—A micrograph similar to Figure 6, in which the tips of the maxillary stylets appear separated, presumably in their feeding position. ×1890.

plants, demonstrated a distinct increase in sap uptake after the first hour of feeding. They interpreted this as evidence that normal feeding did not occur until the phloem was reached. Mittler (1957, 1958) studied the feeding of *Tuberolachnus salignus* on *Salix* stems and revealed that the stylet tips were terminating in the phloem sieve tubes. The stylets of this species, if severed from the feeding aphid, continue to exude sap from the sieve tubes for many hours—a feature which has been made use of in phloem translocation studies. Mittler concluded from his study that aphids may depend for their food supply on the natural high turgor pressure of phloem cells.

Unfortunately there is no data available on the time required by *H. lactucae* to acquire LNYV from *S. oleraceus*, but circulative viruses are characteristically acquired only after several hours, or often days, of vector feeding. With LNYV this period is almost certainly much longer than that required for the stylets to reach the phloem.

Further, it is felt that the predominantly intercellular passage of the stylets through cortical parenchyma tissue of *S. oleraceus* is another indication that these cells are not an important source of virus for the aphid. Although xylem tracheids and the vessels of *Nicotiana glutinosa* have been shown to contain LNYV (Chambers and Francki 1966) it is unlikely that the xylem of *S. oleraceus* is an important site of virus acquisition by *H. lactucae* as only one track of the 159 studied was found to terminate in that tissue. There is therefore little doubt that LNYV is acquired by the aphid from the phloem cells of *S. oleraceus* on which it normally feeds.

V. Acknowledgments

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