ANATOMIC EFFECTS OF CURLY TOP AND ASTER YELLOWS VIRUSES ON TOMATO

ENGRACIA ARGUELLES RASA and KATHERINE ESAU

STUDIES ON PENETRATION OF SUGAR BEET LEAVES BY STYLETS OF MYZUS PERSICAE

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Curly top and aster yellows viruses affect the food-conducting tissue, that is the phloem, of the tomato plant. This tissue undergoes an abnormal increase in number of cells, most of which mature to resemble the food-conducting cells, or sieve elements. The abnormal phloem dies precociously and is crushed. Since the first signs of phloem degeneration occur near normal sieve elements that are first to mature in a given part of leaf, stem, or root, it appears that the viruses move through these elements in their spread through the plant.

The effects of the two viruses are fundamentally similar, but differ in detail. The proliferated phloem shows a more orderly cell arrangement in aster yellows plants than in those affected by curly top. This phloem consists almost entirely of sieve elements in curly top plants, whereas it also contains parenchyma and companion cells in aster yellows plants. The diseased phloem collapses in large, continuous masses in curly top plants, and in small, scattered masses in aster yellows plants. Rod-like crystals, pointed at one end and diamond-shaped in cross section, were found in phloem cells of aster yellows plants, but none was seen in curly top plants.

STUDIES ON PENETRATION OF SUGAR BEET LEAVES BY STYLETS OF MYZUS PERSICAE

The feeding habit of the aphid, Myzus persicae, that transmits the virus of the beet yellows disease of sugar beet, was studied by examining the course of the insect’s mouth parts in sugar beet leaves. Of the 150 penetrations that were identified by the saliva sheaths left by the insects in the leaf tissues, 50 per cent terminated in the phloem tissue, the others in the mesophyll or other parenchyma. The frequent penetration of the phloem by the feeding insects suggests that the insect could release the beet yellows virus into and pick it up from the phloem. This manner of virus transmission would agree with the previously obtained evidence that the virus affects the phloem tissue primarily.
STUDIES ON PENETRATION OF SUGAR BEET LEAVES
BY STYLETS OF MYZUS PERSICAE

K. ESAU, R. NAMBA, and E. A. RASA

INTRODUCTION

The present study was undertaken as a corollary to studies on various aspects of beet yellows, a viral disease the causative agent of which is transmitted by Myzus persicae (Sulzer) and certain other aphids. As judged by the transmission and translocation of the beet yellows virus (Bennett, 1960) and the histologic changes induced in the host (Esau, 1960), the virus is transported through the plant in the phloem but is not strictly limited to that tissue.

According to Roberts (1940), Sylvester (1956), and Bennett (1960), *M. persicae* is more successful in transmitting the virus during long periods of penetration of leaves than during short ones, and Roberts and Bennett suggest that this difference may depend on whether or not the insect reaches the phloem. Roberts (1940) examined 39 stylet penetration tracks of *M. persicae*, 18 produced in 15-minute periods, 12 in 2-hour periods, and 9 in 24-hour periods, and found that only 2 out of 18 reached the phloem in the 15-minute set, 6 out of 12 and 7 out of 9 in the longer-lasting penetrations. Some preliminary studies on the feeding habit of *M. persicae* led Bennett (1960) to conclude that this aphid feeds predominantly in the phloem. To test this concept further, the present study was undertaken.

MATERIAL AND METHODS

Noninfective *Myzus persicae* and healthy sugar beet (*Beta vulgaris* L.) seedlings with the first two foliage leaves partly or fully expanded were used. The insects were made to fast for at least an hour before they were placed on one of the first two leaves. The leaf material on which the insects had fed was prepared for microscopic study by a paraffin-embedding method involving a fixation in formalin-acetic-alcohol (Sass, 1958, p. 15), dehydration through tertiary butyl alcohol, and embedding in paraffin. The sections were cut 10 microns thick, and stained with hematoxylin and safranin. Since the aim was to survey a large number of penetrations, the simplest course was taken: the penetrations were studied by reference to the saliva sheaths left in the tissues by the insect stylets. With the staining combination employed, the sheaths were stained bright red.

The penetrations were obtained by two methods. In one, groups of insects were placed on a single leaf and killed after the desired time interval had passed. The leaf material was then fixed immediately. This material was prepared and collected by Dr. C. W. Bennett in a greenhouse at Salinas, Cali-
Fig. 1. A, transection of leaf showing the track (t) of saliva sheath formed during an approximately 4-minute penetration within the parenchyma of the vein rib (vr). The track is directed toward the vascular bundle (vb) the phloem of which is located at ph. Part of the India ink dot (d) appears on the upper surface of the leaf. B, part of a saliva track (t) in intercellular position in the epidermis, shown in sectional view of the wall. C, part of a saliva track (t) in intercellular position in the epidermis, shown in surface view of the wall. D, saliva track (t) that passed through an epidermal cell. E, part of a saliva track within vein-rib parenchyma. It shows swellings in the intercellular spaces (i) and is smooth within the cell wall (w). At left, above, one arm of the track has broken the wall of a parenchyma cell at b. Ends of the two arms of the track are indicated by letters e, one within cell wall
fornia. The other method consisted of placing individual insects on leaves and letting them terminate the penetration naturally. The aphid, placed in the middle of the leaf blade, on its upper surface, usually moved to the lower side before attempting a penetration. Occasionally it punctured the leaf through the upper surface. The penetration periods were timed with a stop watch while the insects were observed through a magnifying lens. To locate the stylet punctures for processing for microscopy, a dot of India ink was placed gently on the leaf surface opposite the insect as soon as the duration of the penetration exceeded one minute. The ink dot served as a guide not only for collecting the material but also for searching for the saliva track in the sections (fig. 1, A).

An additional experiment was carried out to study the insect's habit with regard to changing the place of penetration and the duration of a single penetration. An aphid was placed in the middle of the upper side of a leaf and was observed through a magnifying glass. The penetrations were timed with a stop watch. When the aphid wandered off the plant, or when it had made a penetration of more than two hours, the observation was terminated.

**PENETRATIONS BY GROUPS OF APHIDS**

In this experiment groups of insects were given access to feeding on a leaf for 5, 10, 20, 30, and 40 minutes, and for 1 and 2 hours. Sample sections of leaves exposed to the insects for 5 minutes (12 leaf pieces) and 10 minutes (4 leaf pieces) revealed no saliva sheaths and these sections, therefore, were not studied further. The longer-lasting penetrations were analyzed in detail.

The penetrations were characterized as shallow, medium, and deep, depending on the number of cells through which the track marked by the saliva sheath extended. The shallow penetrations were 1 to 3 cells in depth (including the epidermal cells), the medium, 4 cells, the deep, 5 cells or more. The deep penetrations had tracks long enough to reach the phloem of the intermediate veins in the mesophyll but not necessarily the smaller, more deeply imbedded bundles, or the larger bundles associated with vein ribs. None of the shallow or medium penetrations had reached the phloem.

The shallow and medium penetrations were excluded from the calculations of the per cent penetrations that reached the various tissues (table 1) because it was assumed that the short tracks may have resulted from short-time probing penetrations. The combined numbers and per cents of the shallow and medium penetrations in the three time groups in table 1 were as follows: for the 20 to 40-minute period, 9 (34.6 per cent); for the 1-hour period, 9 (26.5 per cent); for the 2-hour period, 7 (12.3 per cent); for all time groups combined, 25 (21.4 per cent). The penetration periods of 20 to 40 minutes were combined because of the small number of penetrations available for each. It should be remembered that the timings of penetrations given in table 1 are only approximate because the individual penetrations were not timed in this experiment.

(below), the other in the lumen of a cell (above). F, part of a saliva track (t) in mesophyll. It is intracellular at n, and mostly in intercellular space elsewhere. The pierced cell at n, is partly necrosed, the parenchyma cell at p, in contact with the track, is normal. G, longitudinal section of phloem showing a phloem-parenchyma cell (p), companion cells (c), and a sieve element (s) with plastids (pl). (A, x 145; B, C, x 1,530; D, E, F, x 990; G, x 515.)
The third, fourth, and fifth columns in Table 1 show that, among the deep penetrations, 43.5 per cent reached the phloem. Those that did not reach this tissue were either directed toward the vascular bundles without reaching them, or entered the parenchyma between the bundles. If the penetrations are classified according to whether or not they were directed toward the vascular bundles, almost 70 per cent fall into the class that did reach the phloem or could have done so if they had been extended far enough. Only one out of 92 penetrations ended in the xylem. This was one of the few penetrations that were initiated on the upper side of the leaf. Of the three other penetrations that were initiated on the upper side, two ended in the phloem and one in parenchyma between the vascular bundles.

### Table 1

**Analysis of Deep Penetrations by Stylets of *Myzus persicae***

<table>
<thead>
<tr>
<th>Access time</th>
<th>Number of penetrations</th>
<th>Per cent of penetrations that reached the various tissues</th>
<th>Per cent of penetrations that were directed toward:</th>
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<tr>
<td></td>
<td></td>
<td>Parenchyma</td>
<td>Xylem</td>
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<tr>
<td>20–40 min</td>
<td>17</td>
<td>76.5</td>
<td>...</td>
</tr>
<tr>
<td>1 hr</td>
<td>25</td>
<td>60.0</td>
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<tr>
<td>2 hr</td>
<td>50</td>
<td>46.0</td>
<td>2.0</td>
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<tr>
<td>Total and per cents of total</td>
<td>92</td>
<td>55.4</td>
<td>1.1</td>
</tr>
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</table>

### Individually-Timed Penetrations

Table 2 records the study on the duration, sequence of durations, and number of penetrations for individual *M. persicae* left undisturbed on sugar beet leaves. As can be seen, the feeding activities were highly variable among the aphids, and most penetrations lasted less than 1 minute. In the study detailed in Table 3 a larger number of penetrations were maintained for several minutes. By carrying out a series of 5-minute observations, Sylvester (1954) also noted a variability in the feeding activities of *M. persicae* (on *Brassica juncea* plants infected with *Brassica nigra* virus). We observed that the first two or three penetrations were initiated abruptly, whereas the later ones—even those that were of short duration and were preceded and followed by short-time penetrations—were preceded by light, rapid taps on the leaf surface with the tip of the insect’s beak.

Table 3 details the observations on the penetration tracks formed by individually-timed insects. The penetrations listed in the first column were made either singly by one aphid or several in succession by one aphid. In this experiment the largest number of penetrations by one aphid was four. Of these four, one was not recognized in the sections; the other three were numbers 20, 26, and 41 in Table 3. The first three penetrations by this one aphid were of relatively short duration and were made in the following sequence: No. 26, 9 minutes; missing number, 13 minutes; No. 20, 6 minutes. Then fol-
**Table 2**

DURATION OF INDIVIDUAL PENETRATIONS OF SUGAR BEET LEAVES BY STYLETS OF *MYZUS PERSICAE*

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<th>Aphid number 3</th>
<th>Aphid number 4</th>
<th>Aphid number 5</th>
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<th>Aphid number 7</th>
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* Observation was discontinued before the aphid naturally terminated the penetration.
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<th>Penetration number</th>
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<th>Depth of penetration</th>
<th>Tissues reached</th>
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<td></td>
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<td>Shallow</td>
<td>Medium</td>
<td>Deep</td>
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</tr>
<tr>
<td>Per cent</td>
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<tr>
<td>Per cent for deep penetrations only</td>
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<td>67.3</td>
<td>32.7</td>
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</table>

* Terminated in xylem but passed through phloem.
† The insect was removed after 9.5 hr of feeding because it produced young aphids.
allowed penetration No. 41, which lasted 9.5 hours, after which the insect was destroyed because it entered vivipary. As shown in table 3, the last penetration reached the vascular tissue.

Table 3 as a whole clearly indicates that short-time penetrations rarely reach the phloem. Penetrations 16 and 18 constitute the two notable exceptions. These two penetrations showed no unusual features in the relation of the saliva sheath to the host tissues. Penetration 16 was made in a somewhat older leaf with large intercellular spaces, penetration 18 in a younger leaf with small intercellular spaces. In both, the track was partly intercellular and partly intracellular. Beginning with the second 30-minute penetration (No. 37), the feeding tracks were consistently terminated in the phloem. The proportion of terminations in the phloem for the deep penetrations is slightly higher in table 3 than in table 1. Statistically, however, the difference is not significant: adjusted \( X' = 0.71 \); d.f. = 1; \( P = 0.39 \).

**HISTOLOGIC ASPECTS OF PENETRATIONS**

The relation of the saliva sheaths of aphids to the host tissues has been studied by several workers (see "Discussion," p. 526). The conclusions reached by most workers regarding the manner of entry of the mouth parts into a leaf and their passage and direction through the tissues were confirmed in the present study; but we failed to recognize the penetration of sieve elements.

It is obvious that *M. persicae* feeding on sugar beet leaves does not seek out the stomata. Where penetrations occurred in a stomatal area, the track was made through cell walls or through cells; in one instance it was seen traversing the guard cell itself. Not a single clear evidence of penetration through a stomatal pore was found. Entry through the epidermal cell wall, that is, an intercellular entry (fig. 1, A–C), appears to be more common than one through an epidermal cell (fig. 1, D). The characteristic saliva deposit on the surface of the epidermis (fig. 1, B, D; cf. also Hoof, 1958, and Smith, 1926) was noted in many sections.

The path through the mesophyll, as well as through the parenchyma (fig. 1, A, E) and collenchyma of the vein ribs, was partly or entirely intercellular. If intercellular spaces were present in the tissue, the saliva sheath showed enlargements in those spaces (fig. 1, E at i). Piercing of cells was noted more often in younger than in somewhat older leaves. (All leaves used were relatively young.) Several of the tracks were branched (e.g., fig. 1, E).

In some sections a disorganization of protoplasts of the pierced cells was recognized. The cells sometimes showed an increase in density of cytoplasm (fig. 1, F at n) and a degeneration of plastids, at other times an advanced stage of necrosis (fig. 1, D, epidermal cell). The necrosed material was stained red, but differed from the red-staining saliva sheath in having a somewhat yellowish hue combined with the red. In some pierced cells disintegration phenomena were not recognized (fig. 1, E, at left above), possibly because of technical imperfections. No evidence was found that cells located next to a track, but not pierced by stylets, were affected (fig. 1, F at p).

The saliva sheath showed the characteristics described by Hoof (1958). The sheath was smooth when enclosed between cell walls (fig. 1, B, C, E at w), but it was uneven when located in intercellular spaces (fig. 1, E at i) or within cells (fig. 1, F at n). No difference in thickness was found between
Fig. 2. A, part of a saliva track (t) in phloem in transection. It is intercellular in position, and directed toward a sieve element (s). B, part of a saliva track (t) in phloem in transection. It is intercellular in position, passes through the phloem in contact with a sieve element (s) and a companion cell (c), and terminates in procambium. C, part of a saliva track (t) in mesophyll passing, intercellularly, next to a minor vein in contact with a sieve element (s). This track continued beyond the bundle almost through the entire thickness of the mesophyll. D, part of a saliva track in phloem in longitudinal section with two swellings (t) produced in the two parenchyma cells that were pierced. A sieve element occurs at s. The very tip of the track was bent away from the plane of the photograph. E, part of a saliva track (t) in phloem in transection. The tip of the track is within a parenchyma cell and has dented the wall of another parenchyma cell (p). Two sieve elements appear at s. F, part of a saliva track (t) in phloem in longitudinal section. It is intercellular in position and is superimposed over a parenchyma cell and a sieve element (s). The xylem is indicated at x in B and C. (All × 1,530.)
sheaths that reached the phloem and those that did not. However, many of
the sheaths formed in the experiment with individual aphids (table 3) were
noticeably thicker than others (e.g., figs. 1, A, and 2, C).

A special effort was made to determine the relation of the feeding tracks
to the sieve elements. The total number of penetrations that reached the
phloem in both experiments was 56. Of these, only 33 showed the sheath and
the sieve elements in such a way that their spatial relation could be deter­
dined under an oil-immersion lens. Such a determination is by no means
simple, especially if the sieve elements are narrow. The sieve elements of the
sugar beet are rather narrow cells, particularly in the minor veins (Esau,
1960) in which the feeding tracks so often terminate. However, the charac­
teristic clearness of the contents of the sieve elements and the presence of
sieve-tube plastids (fig. 1, G) help to identify these cells even if the vascular
bundles are cut on a bias.

Among the 33 tracks examined with regard to their course in the phloem,
not a single one terminated in a sieve element. The closest to such a termina­
tion is shown in figure 2, A. Here, the track (t) is intercellular, and ends just
outside the wall of a sieve element (s). In several views the track was seen
in contact with sieve elements. In figure 2, B, for example, the track passed
intercellularly through the phloem directly next to a sieve element (s) and a
companion cell (c), and ended in the procambium. In figure 2, C, the track
is seen flanking, intercellularly, a small vascular bundle, again in contact
with a sieve element (s). This particular track continued beyond the bundle
in the next section, passing almost through the entire mesophyll. In several
views the track was seen to have entered a parenchyma cell next to a sieve
element. Although none of these tracks was satisfactory for photomicrog­
raphy, two are shown. In figure 2, D, the track (t) passed through one
parenchyma cell and entered a second, which was located next to a sieve ele­
ment (s). (The figure does not show the very end of the saliva sheath because
it was bent away from the plane of photography.) In figure 2, E, the end of
the sheath appears within a parenchyma cell next to two sieve elements, and
its point has dented the wall of another parenchyma cell (p). (The shrinkage
of the protoplast in the parenchyma cell p appears to be a result of poor fixa­
tion.) In one section the saliva sheath had traversed a phloem parenchyma
cell and entered a companion cell. Both cells had gum-like necrotic material
next to the saliva sheath.

A view that required extra care in interpretation is shown in figure 2, F.
It gives the impression that the track ends within a sieve element. However,
the relatively smooth appearance of the sheath suggests that it is in an inter­
cellular position. There is no evidence of a break in the wall of the sieve
element such, for example, as is recognizable in the wall of the pierced paren­
chyma cell at b in figure 1, E. The view in figure 2, F, actually shows the
saliva track in an intercellular position superimposed over a parenchyma
cell and a sieve element.

**DISCUSSION**

As judged by the histological aspects of the penetration of sugar beet leaves
by the stylets of *Myzus persicae*, the insect could release the beet yellows virus
into and pick it up from both the phloem and the parenchyma outside the
phloem; it may pierce cells in the phloem and also those outside that issue.
The conclusion of Hoof (1958), that aphids can pick up a virus from the inside of a cell wall, cannot be properly evaluated on the basis of the present study. There is no question, however, that some saliva sheaths are entirely intercellular in position. Another problem yet to be solved is whether or not the degeneration of the protoplasts of the pierced cells has any effect upon virus transmission.

Bennett's (1960) and Roberts' (1940) observations that penetrations of longer duration, that is, penetrations which are likely to reach the phloem, are more effective in beet yellows virus transmission than are the short-time penetrations, and the present confirmation that short-time penetrations rarely reach the phloem, support the concept that penetration of the phloem is important for the success of infection. When placed in the phloem, the beet yellows virus may be expected to be carried through the plant in sieve elements, although the present study furnishes no direct evidence that the insect punctures the sieve elements in feeding on the phloem.

The question whether or not the aphid finds the vascular bundles simply by random probing, as has been suggested for the jassids by Day et al. (1952), cannot be answered as yet. The preference of the insect for the lower side of the leaf, and the frequent selection of veins suggest some sense of direction, but the observation that only about half of the deep penetrations reach the phloem points toward the possibility that the sense of direction is somewhat limited. A related question is concerned with whether the insect feeds only in the phloem tissue or also in the ground parenchyma. Possibly the shallow tracks, especially those that appear to be entirely intercellular, result from probing penetrations and involve no feeding; but there is also the high percentage of deep and prolonged penetrations not ending in the phloem that cannot be properly interpreted at the present state of our knowledge of the feeding habits of aphids.

The tracks formed in host tissues by aphids and many other insects in their search for food have been repeatedly investigated with and without reference to the transmission of viruses. Only one aspect of these studies is reviewed here: whether or not conclusive evidence has been presented that, in penetrating the phloem, an insect actually punctures a sieve element. The assumption is generally made that insects feeding on the phloem do tap the sieve elements. For the aphids specifically it is postulated that they do not suck the contents of sieve elements, but depend almost entirely on the pressure of the sieve-element contents, which forces the sap up the food canal of the stylet (Mittler, 1957). The ingenious experiments involving the severance of aphids from their stylets during the process of feeding, and the collecting of sap exuded from the stylets have enabled workers to carry out some highly significant studies on the phloem sap; and the composition of the exudate suggests that it could have originated in sieve elements (e.g., Mittler, 1957, 1958; Weatherley et al., 1959). With reference to these experiments, as well as with regard to the transmission and translocation of viruses, the determination of the cell tapped by the insect is of great interest.

Published papers dealing with feeding tracks of various insects, reviewed in connection with the present study, reveal that many authors have submitted illustrations clearly demonstrating penetration of the phloem tissue (e.g., Dykstra and Whitaker, 1938; Leonhardt, 1940; Smith, 1926; Tate,
1937). With regard to the puncturing of sieve elements, however, we are given either statements (e.g., Mittler, 1957; Lindemann, 1949; Weatherley et al., 1959), or idealized drawings (e.g., Horsfall, 1923; Rawitscher, 1933–34), or inadequate photomicrographs. In the photomicrograph by Romell (1935), supposedly depicting punctured sieve elements, the identity of these elements is not obvious in the picture, and they are said to be “filled with cytoplasm,” a feature not typical of mature sieve elements. The low-power photomicrographs of sections cut on bias reproduced in the paper by Franke-Grosmann (1937) are even less informative regarding cells penetrated by the aphid (Lachnus pichtae) in its host (Abies). The drawings of Hargreaves (1915) and Pollard (1955), depicting the feeding of a white fly larva, show the stylets of the insect ending in a bundle-sheath cell. Pollard states that “the stylet penetrates a sieve-tube,” but his drawing, obviously, does not confirm this conclusion. Photomicrographs most closely suggesting penetration of sieve elements by insect (aphid) stylets appear in Mittler’s thesis (1954). An entirely certain identification of the cells in which the stylets end is not possible from these illustrations, but one of them shows the tip of the stylet bundle located in an elongated cell, and this tip is free of saliva.\(^6\)

This absence of saliva at the tip of the stylets appears to be significant in relation to the present study in which only saliva sheaths were examined. Conceivably, the insect projects the stylets beyond the saliva sheath during the actual feeding process and penetrates the sieve element without leaving any easily detectable puncture. Such projection of mouth parts beyond the saliva sheath was observed in jassids feeding on a solution through a plastic membrane (Day et al., 1952). In fact, deposition of saliva into the sieve element could possibly interfere with the feeding process by interrupting the flow of sap from the sieve element.

**SUMMARY**

By reference to saliva sheaths left by *Myzus persicae* in sugar beet leaves, over 150 penetrations were studied with regard to their position in the leaf tissues. Some of these penetrations were made by aphids placed in groups upon single leaves and then killed after having had access to the leaves for periods of various lengths. The others were obtained by placing individual insects upon a leaf and timing naturally-terminated penetrations. When penetrations lasted only a short time they usually were shallow. Penetrations of longer durations produced deeper punctures, and of these approximately 50 per cent terminated in the phloem tissue, the others in the mesophyll or other parenchyma. Within the phloem, the saliva sheath may be entirely intercellular or may traverse or terminate in phloem-parenchyma cells or companion cells. The sheath was seen in contact with sieve elements in intercellular position. Outside the phloem the sheath may be entirely intercellular or may penetrate epidermal or parenchyma cells.

\(^6\)Since this review was written, Dr. Martin H. Zimmermann (1961) of Harvard University has succeeded in obtaining convincing photomicrographs showing the stylets of a large aphid, Longistigma caryae, ending in a sieve tube of *Tilia*. As in Mittler’s (1954) figures, the stylet ends are free of saliva in those photomicrographs.
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