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# Studies on *Gomphocarpus physocarpus:* Further Evidence of Preferential Feeding by the Aphid, *Aphis nerii* on the Internal Phloem

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With 19 Figures

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## Summary

Penetration of the stems and leaves of *Gomphocarpus physocarpus* (E. Mey) by the aphid, *Aphis nerii* (B. de F.) was studied with light and phase microscopes. Penetration of the epidermis and ground tissues was largely intercellular, that of the phloem tissues partly intercellular and in part intracellular. In the large majority of penetrations the external phloem was bypassed, the stylet tracks terminating in the sieve tubes of the internal phloem. Of 75 pairs of stylet tips encountered in presumably functional sieve tubes 73 were lodged in sieve tubes of the internal phloem. This confirms observations of a preliminary study which indicated that *A. nerii* feeds preferentially on sieve tubes of the internal phloem. A satisfactory explanation of this preferential feeding has yet to be provided.

## 1. Introduction

As is characteristic of other members of the *Asclepiadaceae*, the shoot system of *Gomphocarpus physocarpus* contains both external and internal phloem. During a preliminary study of the feeding habits of the aphid, *Aphis nerii* on young stems of *G. physocarpus* (Botha *et al.* 1972), it was noted that *A. nerii* apparently feeds almost exclusively on sieve elements of the internal phloem.

A thorough survey of the literature indicated that other studies on the manner and habit of aphid feeding had been made with plants that lacked internal phloem or bicollateral bundles. Consequently, no other observations of preferential feeding by aphids on internal phloem have been recorded.

The principal aim of the present investigation was to obtain more detailed information on the feeding habits of *A. nerii* on *G. physocarpus* and, in the

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process, to obtain further evidence of preferential feeding by the aphid on the internal phloem. Leaf material upon which the aphids were feeding was examined, in addition to stem material.

## 2. Materials and Methods

Colonies of the aphid, *Aphis nerii* (B. de F.) were established on *Gomphocarpus physocarpus* (E. MEY) plants ranging in age from six to 20 weeks and older. Plants with suitably established aphid colonies were selected for light microscope investigation of stylet penetration. The aphids were killed in situ by exposing them to 100% acrolein vapour. Leaves and stem segments to which aphids remained attached, were immediately transferred to FAA (SASS 1958) and were fixed under low vacuum for 24 hours, after which the tissue pieces were diced into smaller, more managable pieces, and dehydrated through an alcohol series. Embedment was in paraffin wax to which 20% (w/w) of ceresin had been added. Sections were cut on a rotary microtome at 12 µm. The sections were variously stained. Safranin-fast green proved to be the most suitable staining combination. With this combination the stylets stained a golden brown colour and the salivary sheath a dark red. Selected sections were photographed under phase and transmitted light.

## 3. Results and Discussion

### *3.1. Brief Description of Stem and Leaf Segments Examined*

As mentioned, the stems ranged in age from 6 to 20 weeks, and older. Both young and mature leaves were examined.

Most aphid feeding occurred on young stems, consisting almost entirely of primary tissues. As seen in Figs. 1–4, the six week old stem consists of a

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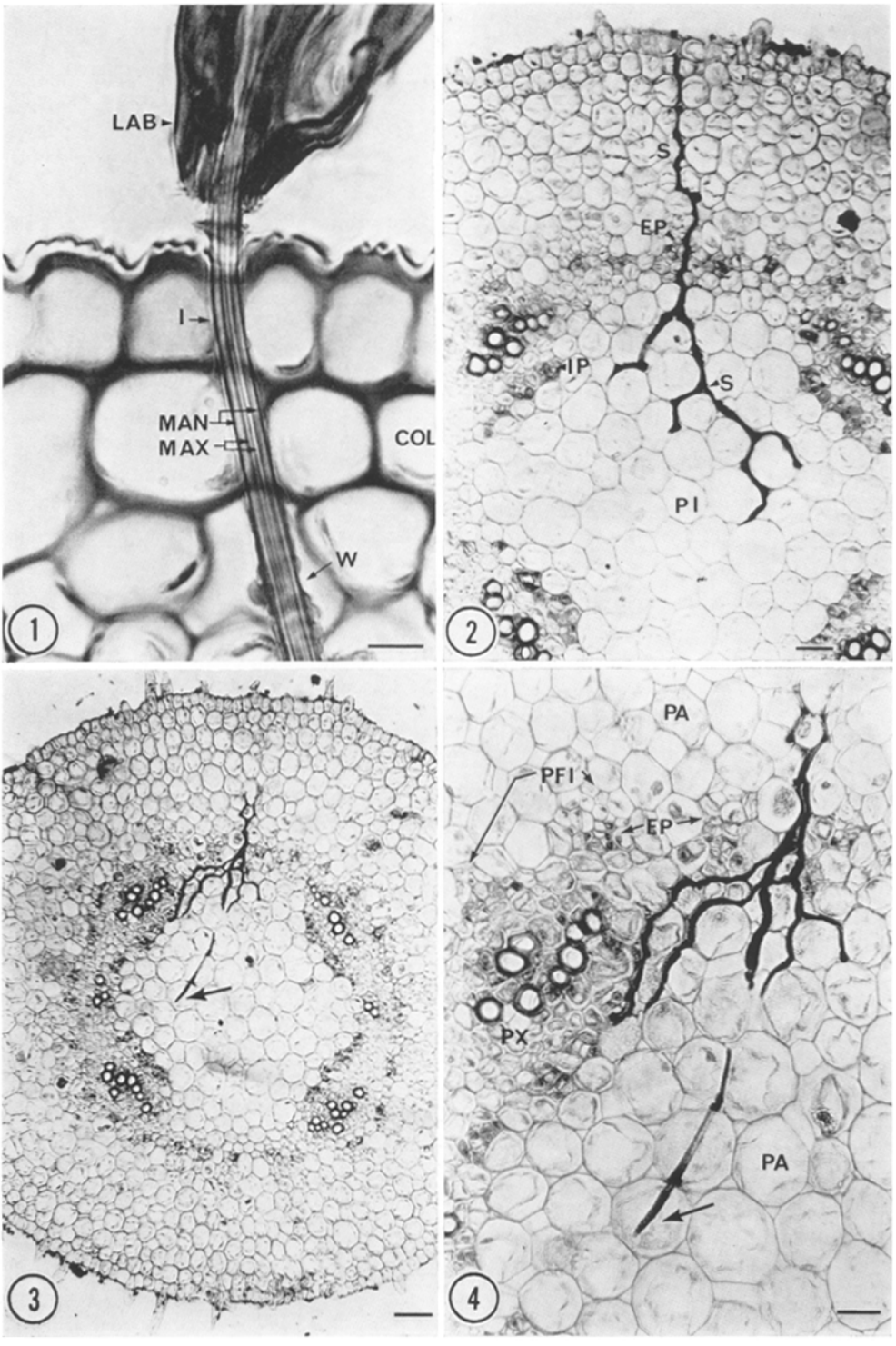
Figs. 1–4. Transections of six week old stem tissue, showing several attempts at phloem probes

Fig. 1. Shows penetration of a stylet group through the uniseriate epidermis and underlying partly differentiated collenchyma. Penetration is predominantly intercellular, except for the lower, partly differentiated collenchyma cell which has been penetrated intracellularly and contains salivary material. *LAB* = Labium, *MAN* = mandibular stylets, *MAX* = maxillary stylets, *COL* = collenchyma, *I* = intercellular penetration, *W* = intracellular penetration. Scale line represents 6 µm

Fig. 2. Shows branched stylet sheath running through cortical tissue and external phloem and terminating after four unsuccessful probes for internal phloem in the pith. *EP* = external phloem, *IP* = internal phloem, *PI* = pith parenchyma, *S* = stylet sheath. Scale line represents 60 µm

Fig. 3. Shows a branched stylet sheath terminating in the internal phloem. The stylets are lodged within the pith (unlabelled arrow). Scale line represents 80 µm

Fig. 4. At higher magnification it can be seen that during the course of penetration, the stylets shown in Fig. 3 passed in close proximity to an external phloem bundle without signs of feeding on these external sieve tubes. *PFI* = immature primary phloem fibers, *EP* = external phloem, *PX* = protoxylem, *PA* = parenchyma, unlabelled arrow = stylet group. Scale line represents 40 µm




Figs. 1–4

uniseriate epidermis, a cortical region of 1–3 layers of partly differentiated collenchyma cells and several layers of parenchyma, a ring of primary vascular bundles of varying age associated with relatively narrow parenchymatous interfasicular regions, and a pith. In the stem of Figs. 1–4 the external and internal phloem is entirely primary; that is, it is procambial in origin. As the stem ages a vascular cambium arises between the metaxylem and external phloem and produces some secondary phloem on its outer face and secondary xylem on its inner face. Primary phloem fibers eventually develop on the outer margin of the vascular cylinder.

In both the external and internal primary phloem the sieve elements and associated parenchymatous elements, including the companion cells, occur in more or less distinct groups, the number of groups per vascular bundle being directly proportional to the size of the bundle. The external phloem bundles contain 2–10 sieve tubes. The internal bundles generally contain more sieve tubes (range 1–20 or more) than the external bundles. Articulate laticifers occur throughout the stems, but are associated mainly with the external and internal phloem.

The larger vascular bundles of the leaf are bicollateral, *i.e.*, they contain both adaxial and abaxial phloem (Figs. 15 and 16). The bundle of the petiole and midrib exhibits a limited amount of secondary growth through activity of a vascular cambium located between the xylem and the abaxial phloem. Differentiation of the adaxial phloem lags behind that of the abaxial, as in petioles of *Luffa cylindrica* (SHAH and JACOB 1969).

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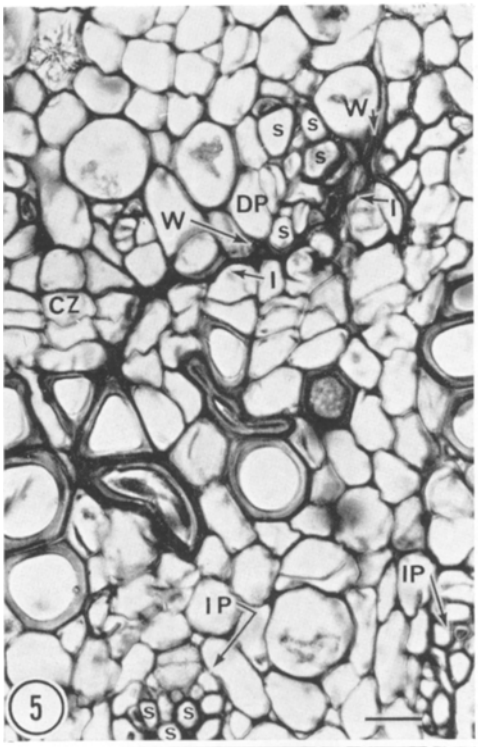
Figs. 5–8. Transections showing stylet penetration of external phloem

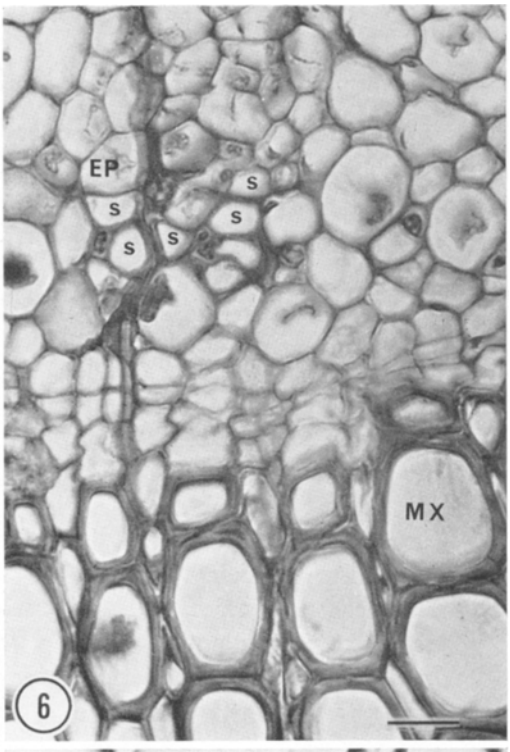
Fig. 5. Shows a stylet track passing both intercellularly and intracellularly, through the external phloem of a young stem. Passage of the stylets through the cambial zone was predominantly intercellular, and through the primary xylem as well. *S* = sieve element, *DP* = dividing external phloem parenchyma cell, *CZ* = cambial zone, *I* = intercellular penetration, *W* = intracellular penetration, *IP* = internal phloem. Scale line represents 20 μm

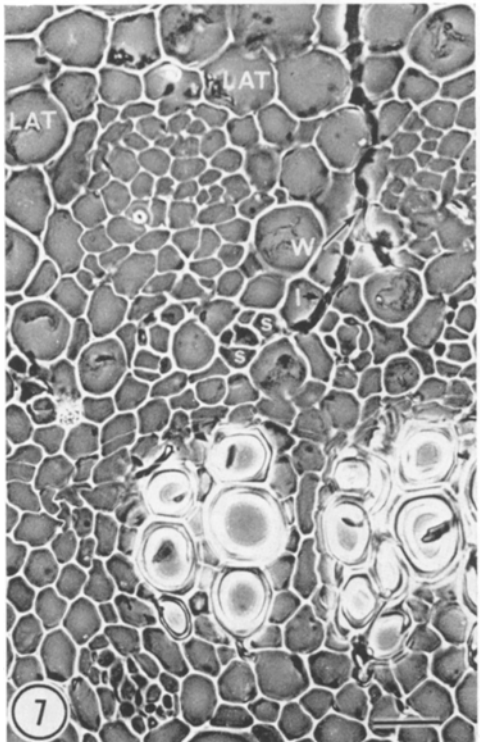
Fig. 6. Shows the predominantly intracellular passage of stylets through the primary phloem fibers, external phloem and cambial zone. There is no evidence for feeding on this bundle, during passage of the stylets. *EP* = external phloem, *MX* = metaxylem vessel. Scale line represents 20 μm

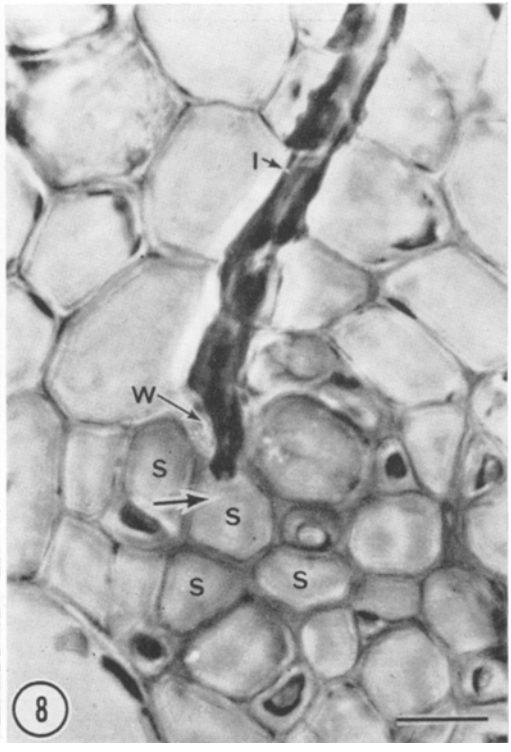
Fig. 7. Phase contrast photomicrograph showing penetration by stylets of an external phloem bundle. Note that the stylet tips are obscured, presumably by sheath material. *LAT* = laticifer, *W* = intracellular penetration, *S* = sieve tube, Unlabelled arrow = punctured sieve tube. Scale line represents 20 μm

Fig. 8. Another example of penetration of external phloem bundle. Passage of the stylets through the surrounding parenchyma was both intercellular and intracellular. As in Fig. 7, the stylet tips are masked by sheath material. *I* = intercellular penetration, *W* = intracellular penetration, Unlabelled arrow = punctured sieve tube. Scale line represents 10 μm




Figs. 5–8

### *3.2. Penetration of the Stem*

Information on the manner of penetration of the stem was obtained primarily by the study of serial sections of sheaths or stylet tracks in host tissues. Seventy-five of the sheaths examined contained stylets, the majority of which were associated with feeding aphids identified as such by the presence of the stylet tips within apparently functional sieve tubes (EVERT *et al.* 1968).

As with most relatively small aphids (see literature cited in AUCLAIR 1963, EVERT *et al.* 1968), penetration of the stem by *A. nerii* was largely intercellular (Figs. 1–4). The stylets entered the stem between epidermal cells and proceeded in a more or less zig-zag pathway along the surfaces of the collenchyma and parenchyma cell walls of the cortex to the vascular tissues and pith (Figs. 1–4). Only occasionally were cortical cells punctured by the aphid's stylets. One such case is shown in Fig. 1, where the lower, partly differentiated collenchyma cell is traversed by the stylets and contains salivary material of the sheath.

Branched stylet tracks were seldom encountered in the cortical region, but were common in and near the vascular tissues and pith (Figs. 2–4). The frequency of branching was greater in the vicinity of the internal phloem bundles and pith (Figs. 3 and 4). As shown in Fig. 2, in the younger stems

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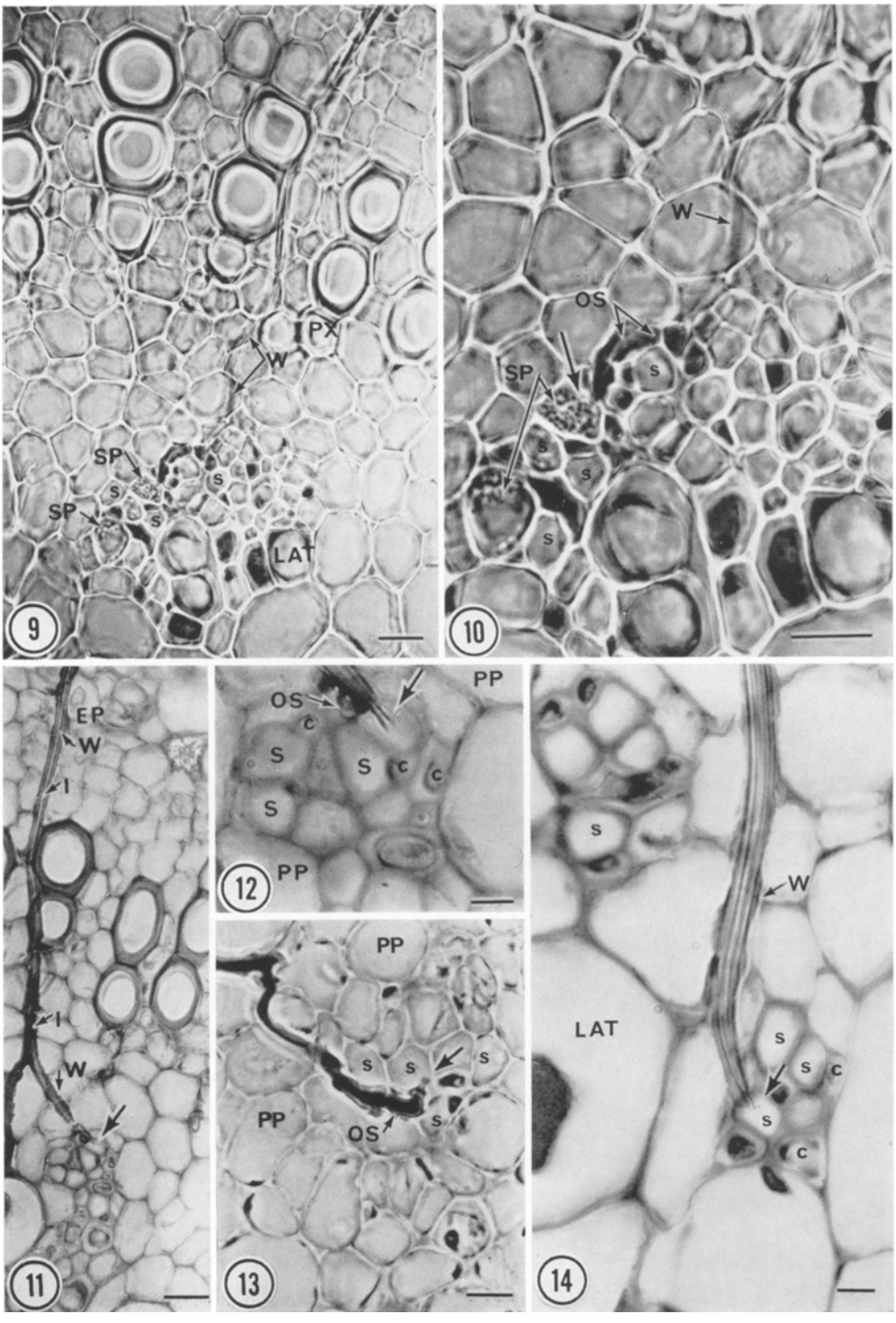
Figs. 9 and 10. Phase contrast photomicrographs showing example of direct penetration of internal phloem by aphid stylets. The passage of the stylets was both intercellular and intracellular. At higher magnification (Fig. 10) it is apparent that the aphid fed sequentially on the smaller sieve tubes, which were subsequently obliterated by sheath material, prior to the penetration of the large metaphloem sieve tube. *W* = intracellular penetration, *PX* = protoxylem, *LAT* = laticifer, *SP* = sieve plate, *S* = sieve tube. Scale line represents 40 and 15 μm respectively

Fig. 11. Illustrates direct penetration of the internal phloem. A metaphloem sieve tube has been punctured by stylets (arrow). Note that external phloem (above) was penetrated intracellularly. *I* = intercellular penetration, *W* = intracellular penetration, *EP* = external phloem, Unlabelled arrow = stylet tips within sieve tube. Scale line represents 25 μm

Fig. 12. At higher magnification, the maxillary stylet tips can be seen to project beyond the salivary sheath and the wall of the sieve tube. The stylets are separated, and apparently free of any muscilaginous deposits. The punctured sieve tube appears uninjured. *OS* = occluded sieve tube, *C* = companion cell, *PP* = phloem parenchyma, Unlabelled arrow = maxillary stylet tips within the sieve tube. Scale line represents 7 μm

Fig. 13. Phase contrast photomicrograph showing predominantly intracellular penetration of the phloem parenchyma bordering internal phloem bundle. Note maxillary stylets (unlabelled arrow) projecting beyond salivary sheath into lumen of sieve tube. As in Fig. 12 the stylets and the lumen of the punctured sieve tube are unoccluded. Details as per Fig. 12. Scale line represents 15 μm

Fig. 14. Shows penetration by stylets of sieve tube of small internal phloem bundle. Note that passage of the stylets through the surrounding parenchyma was mostly intracellular, and that the tips of the stylets are again free of muscilaginous deposits or salivary material. Details as per Fig. 12. Scale line represents 7 μm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Figs. 9–14

the stylet tracks ran more or less directly to the inner tissues. In this case the stylet track traversed an external phloem bundle and ended in the pith where the aphid tried probing in several directions. This resulted in a branched stylet track, no branch of which terminated in the phloem. Although the much branched track in Figs. 3 and 4 approached sieve tubes of the internal phloem (on the left), none were punctured. At the time the tissue was collected, the pertinent aphid apparently was probing deeper into the pith (unlabelled arrows point to the stylet tips in Figs. 3 and 4).

Penetration of the external phloem was appreciably greater in older than younger stems. Although largely intercellular, intracellular penetration of phloem cells of the external bundles was not uncommon, including penetration of differentiating primary phloem fibers (Fig. 6) and of immature as well as mature sieve elements and parenchymatous elements (Figs. 5 and 6). Despite the relatively high numbers of penetrations of the external phloem by aphid stylets in older stems, only two pairs of stylet tips were encountered in sieve elements of the external phloem. In a few cases, stylet-containing sheaths ran more or less directly to the external phloem and ended at sieve tubes (Figs. 7 and 8). However, as in Fig. 8, the tips of the stylets were not discernible within the sieve tubes of these external phloem bundles. Possibly the aphids had withdrawn their stylets during our manipulation of the stem. Nevertheless, even with this taken into consideration, the overwhelming majority of the stylet tips occurred in sieve tubes of the internal phloem.

### *3.3. Penetration of the Internal Phloem*

Passage of the stylets through the cambial zone, where present, and the xylem was mostly intercellular (Fig. 9). Figs. 9 and 10 show a stylet stack traversing the xylem and ending in an internal phloem bundle. The stylets are clearly

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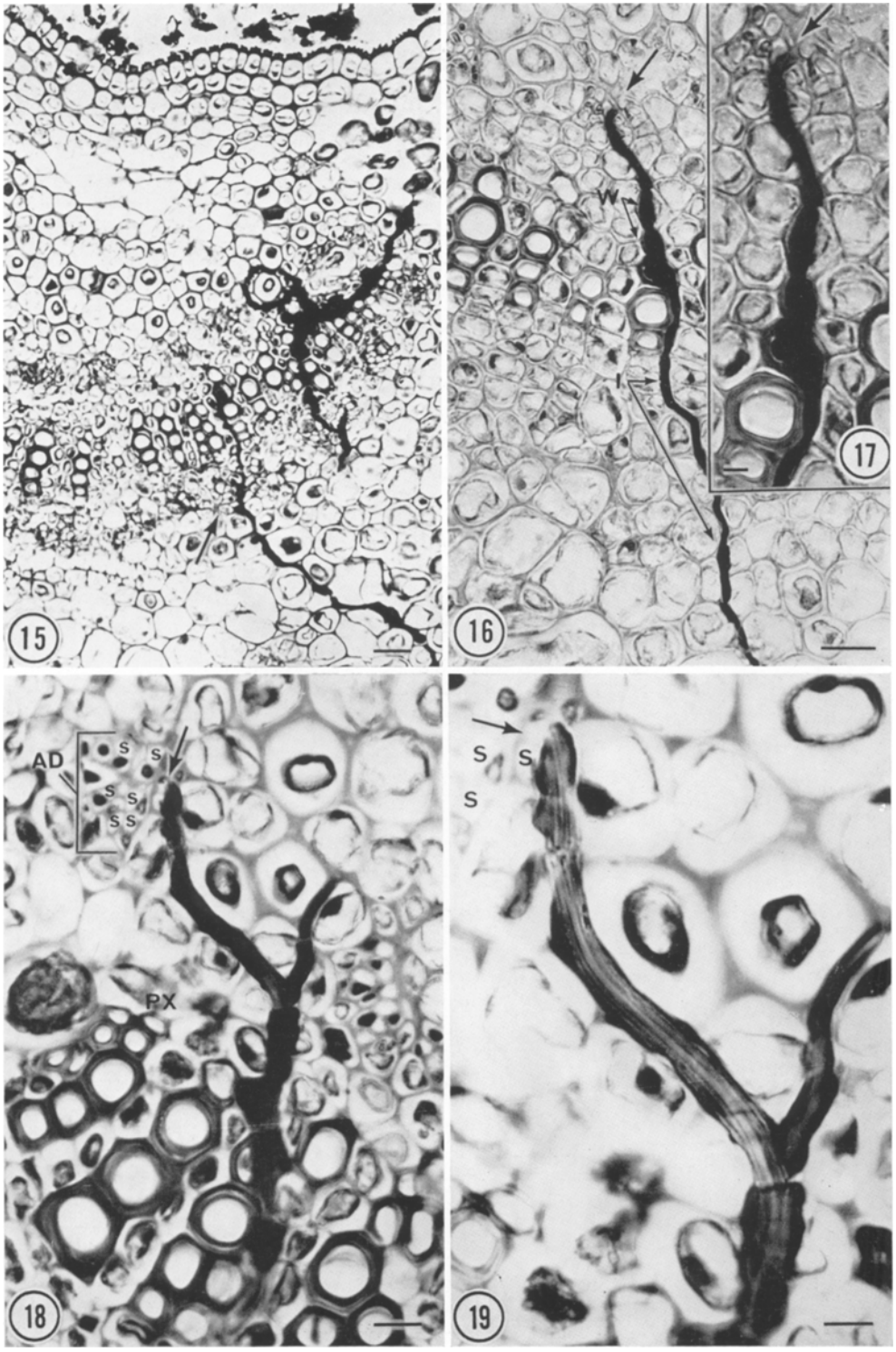
Figs. 15–19. Transections showing penetration of phloem of petioles of mature leaves

Fig. 15. Shows two stylet tracks originating on abaxial surface of petiole. The stylets traversed the abaxial phloem in search of a suitable feeding site. Both end in the xylem. The laticifer on right, above the xylem was completely circumscribed during one of the probes. Unlabelled arrow = adaxial phloem bundle. Scale line represents 30 µm

Figs. 16 and 17. Show passage of stylet track through abaxial phloem and xylem. Note tracheary element obliterated by salivary material. *I* = intercellular penetration, *W* = intracellular penetration, Unlabelled arrow = adaxial phloem probe. Scale lines represent 35 and 15 µm respectively

Figs. 18 and 19. Fig. 18 shows passage of stylets through xylem and termination in adaxial phloem. During this probe the aphid completely missed adjacent adaxial phloem bundle on right. The stylet tips are about even with end of sheath, which terminates at wall of sieve tube. At higher magnification (Fig. 19) the stylets are clearly visible within the sheath. *AD* = adaxial phloem, *S* = sieve tube, *PX* = protoxylem, Unlabelled arrow = probed adaxial sieve tube. Scale lines represent 20 and 10 µm respectively




Figs. 15–19

discernible within the sheath, which terminates at the wall of the punctured sieve tube. Passage of the stylet tips through the sieve-element wall can be seen in Fig. 10. The tips of the maxillary stylets entered the sieve tube below the sieve plate shown here, and are thus not visible.

Whether aphids ingest food through an open or closed sheath has been of concern to a number of investigators (Miles, McLean, and Kinsey 1964, Kinsey and McLean 1967). Results of the present study strongly support earlier reports that aphids feed through an open sheath (Evert *et al.* 1968, Evert *et al.* 1973).

Only the tips of the maxillary stylets penetrated sieve tubes being fed upon. Fig. 11 shows a stylet sheath with stylets traversing the xylem and ending at a sieve tube of a small internal phloem bundle. At higher magnification (Fig. 12) it can clearly be seen that the tips of the maxillary stylets have entered the sieve tube and that they are free of any salivary material.

Figs. 13 and 14 further illustrate the presence of unoccluded maxillary stylet tips in sieve tubes of the internal phloem. In Fig. 13 the stylet tips have barely entered the sieve tube and are still close together. Passage of the stylets through neighbouring parenchymatous tissue was largely intercellular. Within the bundle penetration was in part intercellular and in part intracellular. A sieve tube contiguous to the one containing the stylet tips is occluded with sheath material. In Fig. 14 the stylets can be seen following an intercellular pathway to the punctured sieve tube. Within the sieve tube the stylets are free of any salivary material and are separated from one another, as are those in the sieve tube of Fig. 12.

### *3.4. Penetration of Leaves*

Figs. 15–19 illustrate some aspects of stylet penetration of mature leaves or more specifically, of the petioles of mature leaves.

The probes in leaves were frequently unsuccessful. Two apparently unsuccessful probes can be seen in Fig. 15. Originating from the abaxial surface of the leaf, both probes traversed the abaxial phloem in search of a suitable feeding site and both ended in the xylem. Neither probe reached a sieve tube of the adaxial phloem. It will be noted that, as in the stem, penetration of the petiole was largely intercellular. Note that a laticifer above the xylem on the right was completely circumscribed during one of the probes. This is a reflection of the extreme flexibility of the stylets.

Unbranched tracks frequently bypassed the abaxial phloem, traversed the xylem, and terminated in the adaxial phloem (Figs. 16 and 17). Although penetration of the xylem was largely intercellular, occasional tracheary elements were occluded with sheath material (*e.g.*, the third tracheary element from the bottom in Fig. 17).

Only three pairs of stylet tips were observed within sieve tubes of leaves, and

all of these were in sieve tubes of the adaxial phloem, which is homologous and continuous with the internal phloem of the stem. In other cases, the stylet tips were about even with the sheath, as shown in Figs. 18 and 19. Possibly the aphid withdrew its stylets at the time of sampling. However, Fig. 19 shows that the end of the sheath is still open.

In its initial probe of the region above the xylem, the aphid completely missed the adjacent group of sieve elements.

Most workers have suggested that aphids and other suctorial insects they have studied find their objectives by trial and error (PAINTER 1928, BALCH 1952, DAY, IRZYKIEWICZ, and MCKINNON 1952, EVERT *et al.* 1968). Although *A. nerii* feeds preferentially on the internal phloem of the stem and, apparently on the adaxial phloem of the leaf, it appears that the aphid locates the internal and adaxial phloem with some degree of precision in some instances (Figs. 10, 13, 14, and 16) and in others, largely by chance (Figs. 2, 4, 11, 15, and 18). However, it would seem that the chance location may be more frequent in the younger stem and leaf material examined. This is suggested by the configuration of the aphid tracks within the stem and leaf, by the frequency of branching near the vascular tissues and within the pith, and by the near misses of sieve tubes in both external and internal phloem bundles.

The most intriguing, unanswered question arising from the present study is: "Why do the aphids feed almost exclusively on the internal phloem?" Possibly the internal sieve tubes contain some substance or substances either lacking in the external sieve elements or present in lesser amounts in the latter—substances which are highly desirable to the aphid. This possibility would be difficult to confirm. Both the external and internal phloem are associated with articulate laticifers, the contents of which would interfere with any chemical separation of the phloem sap constituents.

Possibly the external sieve tubes are more easily damaged during penetration by the aphid's stylets than are the internal sieve tubes, rendering the former nonfunctional. However, in this regard, it must be born in mind that the greater majority of stylet tips and tracks bypass the external phloem, without any evidence of penetration of the external phloem sieve tubes.

A further possibility is that greater quantities of assimilates are transported in the internal phloem than the external phloem.

With regard to all the possibilities mentioned above, it must be kept in mind that the aphids more often bypass the external phloem than penetrate it. Hopefully, developmental and ultrastructural studies now in progress will shed some light on this intriguing problem.

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