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Plant-sucking bugs can remove the contents of cells without mechanical damage[1]

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Summary. A mirid and a coreid, feeding on a variety of plant tissues, evacuated the contents of cells up to 3.5 mm from the furthest penetration of their mouthparts. A pectinase occurred in the salivary glands of the mirid and an invertase in those of the coreid, but not vice versa. Cells in the mirid lesions were apparently emptied while the walls retained their shape, whereas coreid lesions showed an immediate inward collapse of cell walls and engorgement of intercellular spaces.
Key words. Heteroptera; plant bugs; plant lesions; saliva; *Helopeltis; Amblypelta;* pectinase; cell walls.

Plant-sucking insects in the Heteroptera are reputed to pierce the cells they suck or vessels they tap. Those that make multicellular lesions and/or feed on seeds have been described as lacerating tissues by vigorously thrusting their piercing/sucking, stylet-like mouthparts back and forth[2–5]; those that feed on vascular tissue have been shown to penetrate individual phloem cells[6]. The investigation reported here, however, has revealed that some Heteroptera drain parenchymal cells en masse without penetrating them.

In Papua New Guinea, a mirid, *Helopeltis clavifer* (Walker), feeding on cocoa pods and shoots and on sweet potato stems, and a coreid, *Amblypelta* sp., feeding on cassava and sweet potato stems, were found to cause melanized lesions, roughly spherical to more elongate depending on the tissue, extending up to 3.5 mm beyond the feeding puncture. Those of *H. clavifer* on cocoa mostly occurred in parenchyma close to or immediately below the epidermis and were visible externally; those of Amblypelta on cassava and of either insect on sweet potato stems were sometimes externally visible but more often lay mostly or entirely between and/or below the vascular tissue and were not then externally visible, although when such an occluded lesion occurred a few centimeters from the tip of a shoot, it would often wither and die.

The insects, once committed to feeding, allowed a lens to be brought almost into contact with the head, and it proved possible to observe closely the movements of the head capsule and labium during feeding. Often, very little such movement was observed, however, and for the reasons outlined below it seemed possible that typically the distance penetrated by the insect's stylets fell far short of the outermost margins of the lesion that subsequently formed.

Penetration of a substrate by phytophagous Heteroptera is by a reciprocating, 'sawing' action of the four stylets, which form a coherent 'stylet bundle'. As the insects work the bundle as a whole into the substrate, corresponding reciprocating movements of the head capsule are visible. The labium, in which the stylet bundle is housed, does not enter the substrate; its segments 'elbow' backwards as the stylets penetrate, and from the angle of the elbow and its conformation relative to the labrum, it is possible to estimate limiting values for how far the bundle as a whole has been inserted[4], as indicated in figure 1.

Such calculation implies a constant length of stylet bundle (i.e. to the opening of the salivary and food canals) hence a possible source of inaccuracy would be the difference between the maximum protraction and retraction of the stylets relative to the head capsule. This distance is reputed to be relatively small, due primarily to the anatomy of the bases of the stylets and of their actuating muscles[7]. In ad hoc experiments, the stylet bundles of the live insects were gently slipped out of the labium and the stylet tips observed under a microscope while keeping the bundle at right angles to the body. In these circumstances the insects were found to perform violent movements of the stylets, identifiable as an attempted retraction cycle[8], and the maximum distance between any two stylet tips was always less than 0.1 mm in *Amblypelta,* the larger of the species under observation, and possibly no more than 0.02 mm in *Helopeltis.* Any attempt to increase this relative displacement of the stylets by direct manipulation in living or recently dead insects resulted in breakage of the stylets or their attachment.

From these observations, it was concluded that, based on measurements of an individual insect, a geometrical estimate of the maximum exsertion of the stylets during the time the insect was observed feeding could be made as illustrated in figure 1, subject to an *underestimate* of at the very most 0.15 mm for *Amblypelta* and 0.05 mm for *Helopeltis.* Fortunately in relation to the purpose of such estimations, errors considerably greater than these would not have invalidated the conclusion that the lesions formed by the insects usually extended some mm beyond the furthest possible penetration by their stylets.

Thus, when *Helopeltis* fed on previously unattacked cocoa pods, the stylets were typically estimated to penetrate more or less normal to the surface and no further than 0.3 mm deep, yet a hemispherical lesion developed thereafter with a diameter at the surface of between 3 and 4 mm. Deeper stylet penetrations of pods were sometimes observed, most often into those already scarred by previous lesions, but occluded, spherical lesions could then be found subsequently, a few millimeters below the surface of the pod. On cocoa stems, *Helopeltis* again made insertions of less than 0.3 mm, as a result of which lesions developed up to 7 mm axial to the stem and about 3 mm wide.

Amblypelta, when feeding on young sweet potato stems and cassava tips a few mm in diameter, was typically estimated to penetrate up to 0.6 mm, and subsequent sectioning of the stem revealed a lesion in the live pith parenchyma at about that depth, but extending a further 3–4 mm in both directions up and down the stem.

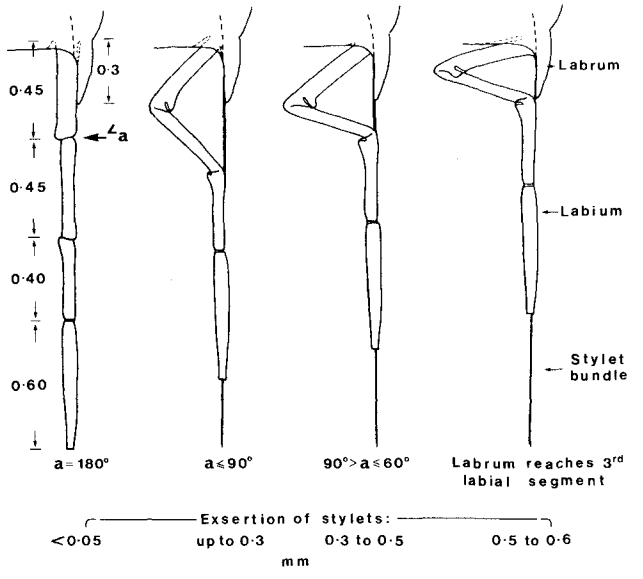

Figure 1. Diagram indicating a basis for estimating the exsertion of stylets of a heteropteron. Figures refer to typical lengths in mm of labrum and labial segments and of exposure of stylet bundle of a specimen of *H. clavifer*. Although variations of anatomical measurements up to ± 0.1 mm were observed between individuals, for any one observation, the accuracy of estimation of maximum possible exsertion at the appropriate limiting configuration was estimated to be ± 0.05 mm for *Helopeltis* and ± 0.15 mm for *Amblypelta* – see text.

Initial penetration was accomplished by either insect within a minute, and they could be observed feeding from the one site for an hour or more thereafter with no further apparent movement of the head. When lesions caused by either bug were visible from the surface, a 'water-soaked' area became apparent within a few minutes of commencement of penetration of the tissues. This area subsequently became melanized to a degree dependent on the tissues concerned. Mirid lesions on cocoa pods became especially intensely melanized, and a brown ring was just perceptible at the edge of the water-soaked area within an hour and spread inwards and intensified over the next 24 h. When both insects made lesions in comparable tissue, e.g. sweet potato, those of the mirid possibly became somewhat more melanized than those of the coreid.

Apart from darkening, the histological features described below were discernible in all lesions within 20 min, although they were most clearly observable after the insects had been feeding for an hour or more. Tissues for sectioning were cut from the plant into Carnoy's fixative, usually within an hour of cessation of feeding; some lesions a day or more old were also fixed for comparison. Hand-cut sections, about three cells deep were found to give the most micro-anatomical information; they were routinely stained with safranin and light green.

All the insects made an intracellular stylet track, typically straight and unbranched. That of the coreid was marked by a solidifying component of the saliva, the 'stylet sheath'[5], which was readily detectable in sections (fig. 2). The sheath formed a channel around the path of the stylets, filling most of the rest of the punctured cells; both sheath and the few remains of the damaged cells became melanized but adjacent cells were apparently little affected by formation of the track. Mirids do not produce a coherent stylet sheath[2]; some solid deposit was detected in the stylet path, but the track was marked mainly by intense melanization of the remains of damaged cells.

For both species, the stylet track was readily distinguishable from the lesions caused by feeding per se, for the melanized contents that remained in cells contributing to the stylet track contrasted strongly with the seeming emptiness of the cells within the bulk of a 'feeding lesion', such as described below. Indeed, a track could sometimes be traced to its end within a newly formed, uncollapsed feeding lesion, presumably because the processes leading to the characteristic appearance of the track had been sufficiently advanced when feeding proper had begun.

All tracks formed in previously undamaged tissues terminated in a layer of parenchyma cells that, in unaffected adjacent tissue, contained starch and other granular reserves substances. The feeding lesions consisted of cells the contents of which had apparently been removed. Such lesions typically extended several mm beyond the end of an identifiable stylet track, and no evidence could be found (e.g. in cleared, hand-cut sections, a few cells thick) that the cells beside or beyond the stylet path had been punctured, confirming the impression gained from head movements that the stylets had not extended to the margins of the lesion subsequently observed. The lesion formed by the mirid during an hour of feeding on any tissue consisted of apparently empty and/or plasmolyzed cells into which melanizing substances had begun to diffuse, thereby darkening cell walls and any substances lining them. In cocoa pods, the outer margins of day-old lesions and especially cells immediately below the epidermis had clearly retained the most cell contents and were the most strongly darkened; in cocoa stems, where mirid lesions were mostly confined to a layer only a few cells deep between the epidermis and the vascular tissue, the cells appeared empty with more or less uniformly darkened cell walls. In day-old mirid lesions, the cell walls had clearly retained their natural outlines, but when tissues containing damage several days old were sectioned, the skeletal remains of tissues within the lesions appeared to have become thinner and fragile; they readily disintegrated when sections were hand-cut.

The cells within lesions caused by the coreids, on the other hand, often appeared to have collapsed during formation of the lesion. In young sweet potato stems, especially, there was evidence that the cell walls swelled initially; the cells within the body of the lesion had a 'squashed' appearance due to shrinkage of the contents, seemingly with a compensatory enlargement of cells at the margin of the lesion, while intercellular spaces had become engorged (fig. 2). As tissues af-

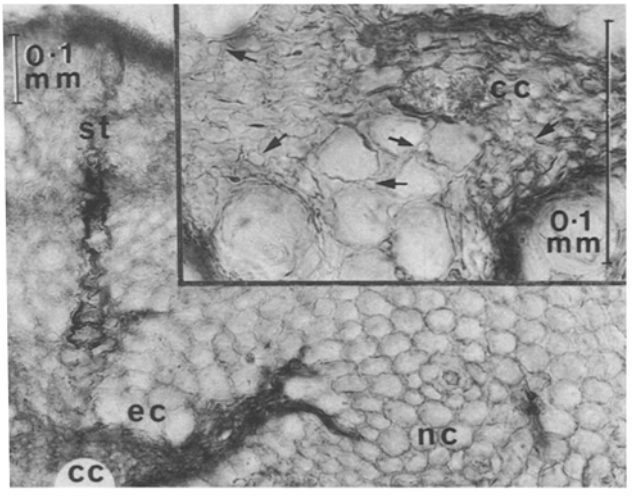

Figure 2. Hand-cut section of tissue of sweet potato after recent feeding by *Amblypelta* sp.; cc, collapsed cells; ec, enlarged cells peripheral to lesion; nc, normal cells; st, stylet track. Inset: part of a lesion at higher magnification; arrows indicate some of the engorged intercellular spaces.

fected by the coreids shrank, either that section of stem sank inwards or, in older, more substantial tissue, the necrotic cells pulled away from those immediately surrounding so that after several days the lesion was marked by a cavity, within which a darkened 'stroma', representing the original tissue, was sometimes distinguishable.

Mirids are known to possess a salivary endopolygalacturonase ('pectinase')[9]. As a simple microviscosimetric test of pectinase activity, the rate of flow (under a standard pressure difference) of microliter quantities of substrate was timed along a horizontal capillary. The substrate used was 3% citrus pectin in phosphate buffer containing salivary gland homogenate (one gland per 30 µl substrate). The extracts from *Helopeltis* reduced the viscosity of the substrate until it was indistinguishable from that of water within an hour at all pH values tested (5–8), but no activity could be found in *Amblypelta*. No cellulase (using a similar technique with methyl cellulose as substrate) was discovered in either mirid or coreid.

As a simple test for invertase, unbuffered 1% sucrose containing salivary gland homogenate was incubated for 2 h at room temperature (about 30 °C), and a drop on paper tested with silver nitrate reagent[10]. Homogenate in distilled water and substrate alone were the controls. Invertase was clearly demonstrated in the salivary glands of *Amblypelta* but none was indicated in those of *Helopeltis*.

Amylase was tested by incubating 1% 'soluble starch' in 1% sodium chloride with gland homogenate, and testing the solution with iodine solution, followed by hydrogen peroxide (because of the strongly reducing properties of the salivary glands of some Heteroptera[5], a falsely positive result may be given unless the oxidizing agent is added.) Neither insect was shown to possess a salivary amylase; minute quantities of human saliva provided a positive test of method.

The injury caused by mirids to their food plants, including nonmechanical spread of symptoms, has been ascribed to their salivary pectinase[11], and perhaps this enzyme contributes to any histological differences of the lesions of *Helopeltis* from those of *Amblypelta*, but for both insects there remains the problem of how they remove the contents of unbroken cells, over 3 mm away from the opening of the stylet bundle, within tens of minutes.

In this, the method of feeding of *Helopeltis* and *Amblypelta* clearly differs from that of e.g. the pentatomid investigated by Hori[4], the lesions of which are formed by an irregular 'assemblage of radial, branch-like stripes' of cells individually pierced by the stylets, the dimensions of the lesion being determined by the maximum reach of the stylet bundle.

Zweigelt[12], considered that the 'salivary secretion' (at that time identified as the stylet sheath) of gall-forming aphids was osmotically active and created a flow of nutrients towards the insects' stylets; he also mentioned release by the insects of amylase and a toxic 'aphidolysin'. Kunkel[13] describing the feeding of Homoptera on parenchyma thought that they might continue to suck from the vacuole of a single cell thereby drawing on an inflow of nutrients from surrounding tissues. The phenomena described in the present work appear to be either far more rapid or on a much larger scale than those alluded to by either author, however; certainly the results of feeding of *Helopeltis* and *Amblypelta* would be difficult to ascribe simply to diffusion of solutes to a single cell or locus, unaugmented by some more pervasive stimulus.

The 'water-soaked' region that is initially formed presumably indicates that some component of the saliva of the insects is injected, rapidly infiltrating the intercellular region. In the mirid, this may well be aided by action of the salivary pectinase on the cell walls, but such an explanation will not do for the coreid. Moreover, the main barriers to diffusion of solutes in plants are cell membranes rather than cell walls, and it seems likely that the diffusion of solutes from physically unbroken cells that takes place within minutes in the lesions of either insect is due to more than impairment of the integrity of the walls alone.

Perhaps, for both mirid and coreid, release of hydrolytic salivary enzyme results in the liberation of osmotically active substances into the intercellular space, causing an outflow of liquid (containing nutrients) that is sucked back from the intercellular space from time to time by the insect. The swollen intercellular spaces in the *Amblypelta* lesions is consistent with such a possibility and its biochemical and biophysical aspects are at present under investigation in this laboratory.

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Acid rain affects egg-laying behavior of apple maggot flies

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Summary. The tephritid fruit fly, *Rhagoletis pomonella,* is less likely to attempt oviposition in host fruit that have been exposed to acid rain or to a simulated acid rain solution (pH < 3.8). Electrophysiological data suggest that acid rain residue on the fruit surface may interfere with the sensory mechanisms that the fly uses during recognition and acceptance of host fruit.

Key words. Acid rain; apple maggot fly; host plant selection; oviposition; pollution; *Rhagoletis pomonella.*

How acid precipitation affects insects and insect-plant interactions is poorly understood, although an increasing number of reports cite complex and varied impacts of acids on plant[2], water, and soil systems[3]. During field tests, we observed per-