

Stylet course of lace bug *Stephanitis typica* (Distant) in coconut leaf

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Abstract. The lace bug *Stephanitis typica* feeds on coconut leaflet from its abaxial side. It inserts its stylet through stomata and sucks the contents of the coconut foliage. Besides this intracellular feeding, the stylet also ruptures the walls of the cells traversed in its course to reach the vascular bundles. The stylet tip in such cases terminates in phloem, thereby suggesting the ability of the bug to acquire the phloem-bound mycoplasma-like organisms, constantly associated with coconut root (wilt) disease in India.

Keywords. Lace bug; *Stephanitis typica*; stylet course; vector; mycoplasma-like organisms; coconut root (wilt) disease.

1. Introduction

Attempts to transmit coconut root (wilt) disease through lace bugs *Stephanitis typica* on the basis of their being the most abundant insect visitors of coconut foliage yielded positive result when they were inoculated on apparently healthy bearing palms in the open field (Nagaraj and Menon 1956; Shanta *et al* 1960) and on coconut seedlings grown in steam-sterilized soil held in cement tubs under an insect-proof house (Shanta *et al* 1964). Solomon *et al* (1983) demonstrated the presence of mycoplasma-like organisms in coconut root (wilt) affected palms and their absence in disease free palms. Since lace bug was abundant on coconut foliage and also it has been incriminated as vector of root (wilt) disease, it was felt that it would be useful to study the stylet course of lace bug in coconut leaf.

2. Materials and methods

2.1 Fixing feeding insects in situ

Lace bugs collected from field were enclosed in 15 × 3 cm test tubes with muslin cloth covered cotton plugs and starved for 16 h. They were then provided with leaflets freshly removed from coconut palm with their distal 30 cm cut and bases dipping in water held in glass jar. Each tube contained about 50 insects. When they settled on the abaxial side of leaflets and started feeding in about half an hour, the leaflets were gently cut in level with the cotton plug taking care not to disturb the feeding bugs. The tube was held in a canister and slowly lowered into a Dewar vessel containing liquid nitrogen and retained there for 2 min. About 30% insects, fixed in the feeding position with their stylets inserted into the lamina (figure 1A) formed the material for study.

2.2 Transections

Lace bugs fixed in feeding position by the cold immobilization technique were

transferred along with coconut leaflet bits to phosphate buffered glutaraldehyde fixative (0.05 M phosphate buffer + 2.5% glutaraldehyde + 0.17 M sucrose adjusted to pH 7.4 with NaOH). After 24 h the tissues were washed in buffer and dehydrated in graded alcohol series: 25, 50, 75, 95 and 100% (two changes) for 15 min each, at 4°C. They were further passed through two changes of 100% acetone (30 min each) at room temperature. Infiltration was effected with acetone-resin mixture and in two changes of resin, in a specimen rotator. The specimens were carefully embedded in fresh Spurr's resin and allowed to polymerise at 70°C in an oven for 24 h. Semi thin sections were prepared from the embedments with LKB Ultratome III. The sections collected over a microscope slide in 10% acetone were dried and stained in methylene blue-Azure II and basic Fuchsin (Humphrey and Pittman 1974).

2.3 Epidermal peelings

Peelings of abaxial epidermis were prepared from segments of coconut lamina with the lace bugs killed in feeding position by fixing in 2% glutaraldehyde and paraformaldehyde at 4°C for 24 h. The samples were then cleared by boiling for 10 min in dilute aqueous nitric acid (1:4) containing 2% potassium chlorate. The dermal layers were peeled apart in water with forceps. The mesophyll cells were swept aside with a camel hair brush. Fixed segments were also dechlorophyllated and made translucent by immersing in boiling lactic acid-phenol-glycerine (2:2:1) for 15 min and subsequently allowed to stay in the clearing agent till the desired degree of clarity was obtained.

3. Results and discussion

Figures 1B and C (epidermal peelings) and figure 1D (transection of coconut leaflet) show that the lace bug inserts its stylet through stomata, which are abundantly present in linear rows on the lower epidermis of coconut leaflet. Based on the absence of any mark of injury to the abaxial epidermis, Mathen *et al* (1979) had suggested stomatal entry of stylet. Johnson (1937) had demonstrated the probe of the tingid *Stephanitis (Leptobyrsa) rhododendri* Horv. through stomata of rhododendron leaf. Pollard (1959) also upheld the intercellular insertion of stylet by the lace bug *Urentius aegyptiacus* Berg. on egg plant leaf. Comparing the paraffin sections of normal leaflets and those fed by lace bugs, Mathen *et al* (1979) pointed out rupture in the cell walls of mesophyll tissue resulting in cavities immediately inner to the lower epidermis and draining of the contents of palisade cells which, however, retained their shape. They suggested a cell to cell penetration of the stylet before it ended in the vasculature. The depth of stylet penetration is up to 600 µm. The maximum thickness of coconut leaflet measured by us is around 400 µm. The insect is therefore theoretically capable of reaching any tissue across the thickness of the coconut leaflet. Figure 2A illustrates the curved stylet track searching for the vascular bundle, breaking the cell walls. The course of the stylet directed straight towards a vascular bundle is illustrated in figure 2B. Each coconut leaf bears several leaflets on either side of a central rachis. Each leaflet has several main and diminutive vascular bundles running parallel with occasional transverse commissures. The smaller bundles are oriented towards abaxial region and the phloem tissues are also in this direction. Access to vascular bundles for the bug is therefore not

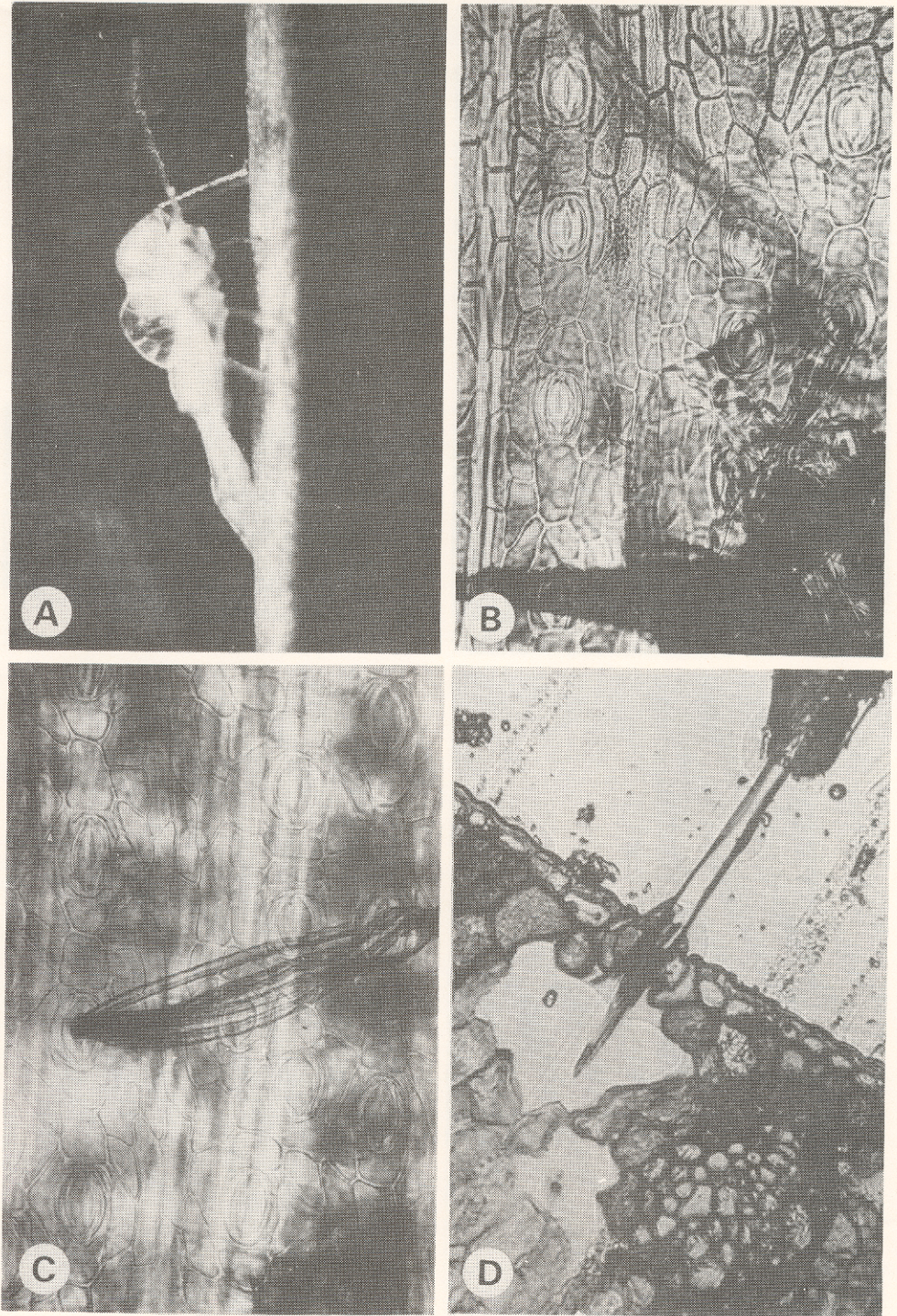


Figure 1. A. *S. typica* in feeding position on coconut leaflet with its stylet inserted ($\times 20$). B and C. Abaxial epidermal peelings of coconut leaflet showing entry of insect stylet through stoma (B, $\times 320$; C, $\times 400$). D. Transection of coconut leaflet showing insertion of insect stylet through stoma ($\times 400$).

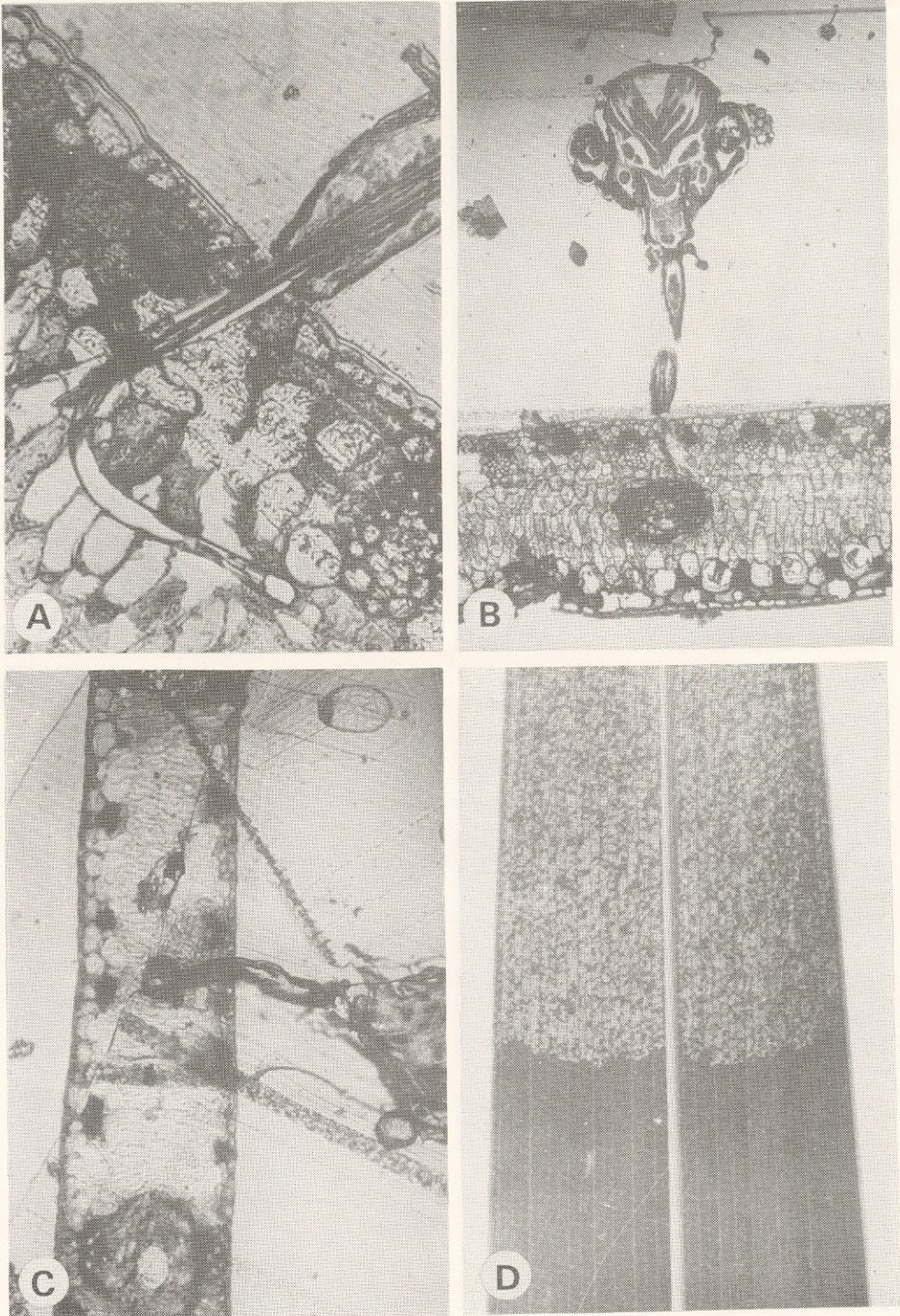


Figure 2. **A.** Transection of coconut leaflet illustrating the curved course of lace bug stylet through mesophyll to reach vascular bundle ($\times 420$). **B.** Lace bug stylet directed straight towards a vascular bundle ($\times 120$). **C.** Termination of stylet in phloem cell in transection of coconut leaflet ($\times 90$). **D.** Permanent marks produced by the lace bug on the adaxial side of coconut leaflet as a result of feeding from abaxial side ($\times 1.5$).

difficult. The termination of the stylet tip in phloem (figure 2B,C) establishes the ability of the lace bug to reach the tissue. There is thus the possibility that the bug may be able to acquire the phloem-restricted mycoplasma-like organisms. Acquisition of the organisms has been evidenced by the detection of mycoplasma-like organisms in salivary glands of lace bugs examined under electron microscope 18–23 days after they were initially allowed to feed on diseased palm for 5 days and the absence of the organisms in bugs from disease free areas not allowed to feed on diseased palm (Mathen *et al* 1987). The feeding marks left on the adaxial side of the coconut leaflet (figure 2D) suggest that the lace bug does not exclusively feed on phloem, but also feeds on palisade cells. A similar situation is reported in the case of *Piesma quadratum* (Fieb.), which feeds intracellularly on individual parenchyma cells of the leaves of sugar beet, producing visible grey or brown spots but has the stylet terminating in the phloem and hence transmits the phloem-limited leaf curl virus (Proeseler 1980).

Mycoplasma-like organism diseases in plants are transmitted mostly by leafhoppers and planthoppers. However, transmission of pear decline by *Psylla pyrisuga* Foester and proliferation disease of carrot by *Trioza nigricornis* Foester (Psyllidae) (Kaloostian 1980); paulownia witches' broom by the stink bug *Halyomorpha mista* Uhler (Pentatomidae) (Shiozawa *et al* 1979); sugar beet savoy disease in north America, now reckoned as of mycoplasma-like organism etiology (Harris 1979) by *Piesma cinereum* (Say) and sugar beet latent rosette disease in German Democratic Republic, caused by rickettsia-like organism confined to phloem cells transmitted by *Piesma quadratum* (Fieb.) (Piesmidae) (Proeseler 1980) gives instances in which Hemiptera other than Auchenorrhyncha transmit the mycoplasma-like organisms and rickettsia-like organisms of plant diseases. *Stephanitis typica* (Tingidae), close to Piesmidae in taxonomic status is an addition to the list of bugs other than the conventional groups of leafhoppers and planthoppers as vectors of mycoplasma-like organisms in plants.

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