

ELECTRON MICROSCOPIC EVIDENCE ON THE ROLE OF *STEPHANITIS TYPICA* (DISTANT) AS VECTOR OF COCONUT ROOT (WILT) DISEASE

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Root (wilt) disease, the century-old debilitating malady of coconut, was suspected to be of virus origin for about three decades¹. Based on experimental evidence²⁻⁴ and observational data⁵⁻⁷, the lace bug *Stephanitis typica* was considered transmitting the disease. But when mycoplasma-like organisms (MLOs), reported to be associated with tissues of diseased palms only⁸ were also included among possible etiological agents, the vector role of lace bug required a reconsideration from the view point of its ability to acquire and sustain the organisms. Electron microscopic studies were therefore carried out to investigate the presence of MLOs in the insect tissues and to find out if it picked up the organism from the phloem of coconut leaflet.

Batches of 25-30 adult lace bugs collected from field were caged in long, loose muslin cloth bags *in vivo* on leaflets of diseased coconut palms known to harbour MLOs. They were collected back after five days. The survivors were maintained *in vitro* on detached leaflets freshly removed from symptomless palms. The bases of these leaflets dipped in water held in glass jars. Fresh leaflets were provided on alternate days. Every day, as long as the insects could be maintained under laboratory conditions, lots of five insects were fixed in 2% glutaraldehyde and paraformaldehyde⁹ in 0.1M cacodylate buffer pH 7.4 at 4° C; post-fixed in 2% osmium tetroxide in 0.1M cacodylate buffer, dehydrated in alcohol and acetone series and embedded in Spurr's resin. Serial ultrathin sections were made in the anterior region covering the brain and salivary gland tissues using LKB ultratome III. Sections were stained with uranyl acetate and Reynold's citrate¹⁰ and examined in a transmission electron microscope (Carl Zeiss EM 109 R).

All the three lace bugs in each of the acquisition plus incubation combination periods of 18, 19, 20, 21, 22 and 23 days revealed large number of MLOs (figure 1) in serial sections through their salivary gland and brain tissues. The spherical bodies, surrounded by distinct unit membrane, were about

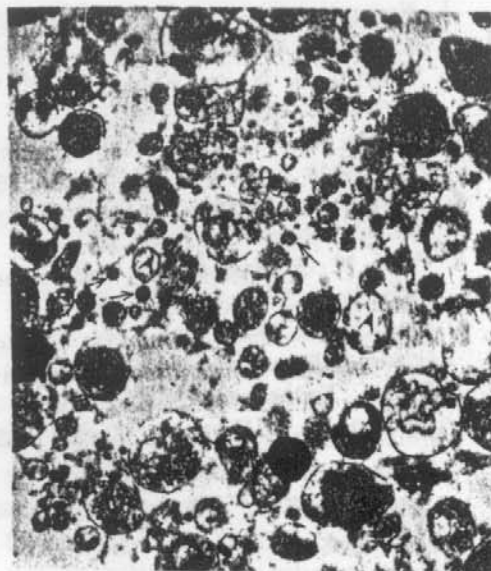


Figure 1. Mycoplasma-like organisms in salivary gland of lace bug: *Stephanitis typica* (arrows: elementary bodies; arrowheads: large forms).

350-500 nm in diameter. Two forms of bodies were observed. The smaller ones were spherical and often filled with electron dense materials; they are probably elementary bodies. The large forms had distinct median area, containing large fibrillar nuclear material and peripheral ribosomes. MLOs were absent in salivary glands of two lace bugs each collected from two disease-free locations: Kasaragod and Minicoy (Lakshadweep).

A previous report of the occurrence of MLOs in the haemolymph and fat bodies of an insect vector in India is from the leafhopper vector of brinjal little leaf¹¹. Although MLO diseases are mostly transmitted by leafhoppers and plant hoppers and rarely by psyllids, there are instances in which members of Piesmididae, taxonomically close to Tingidae to which *S. typica* belongs, transmit a phloem-bound virus disease, an MLO disease and an RLO-induced disease in sugar beet^{12,13}. The detection of MLOs in salivary glands of lace bugs more than a fortnight after the initial access to leaflet from disease-affected coconut palm offers evidence of the bugs acquiring the MLOs, sustaining their multiplication and eventually effecting their occupation in salivary gland.

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