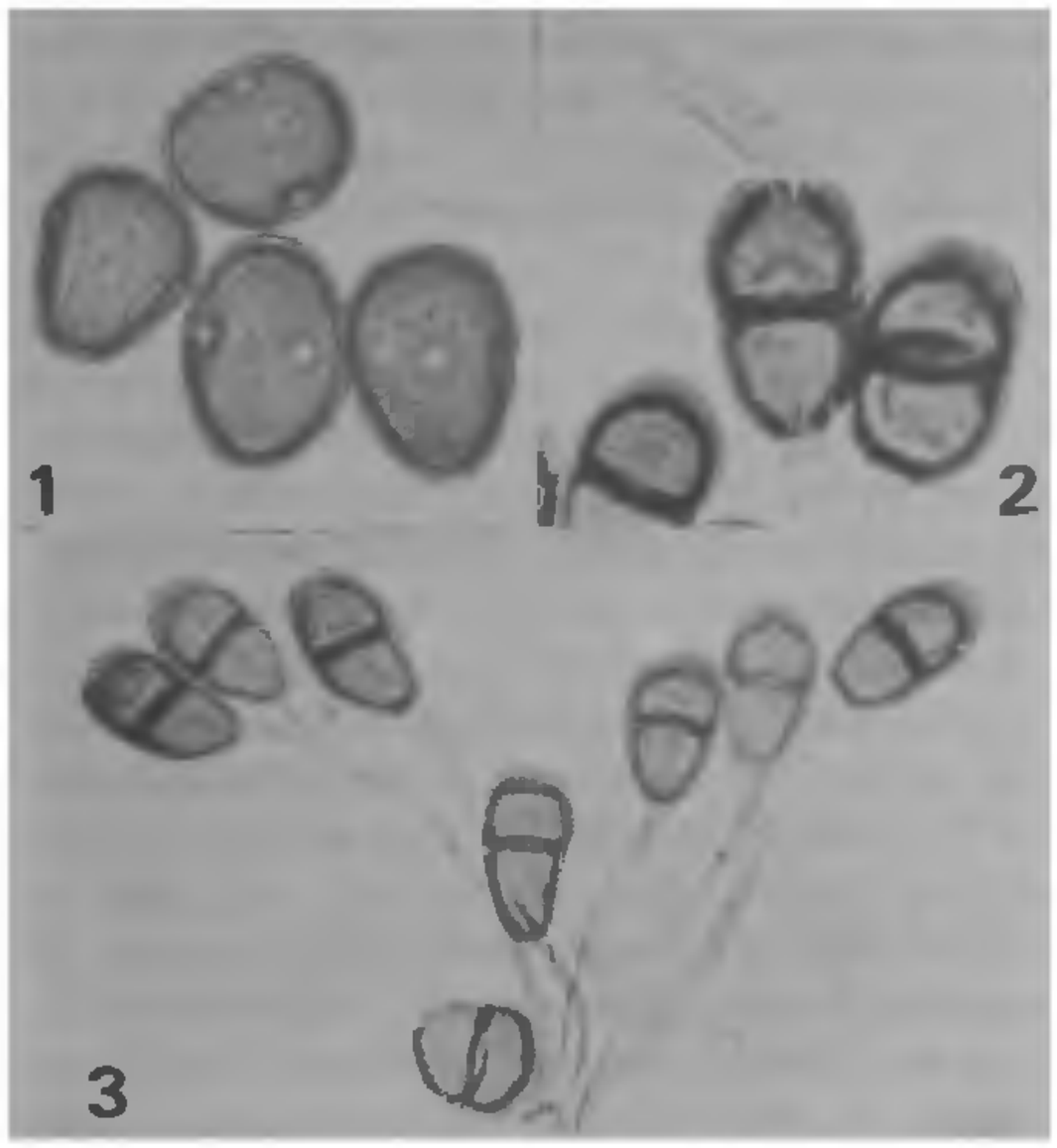

Figures 1–3. Photomicrographs of *Puccinia hyderabadensis*. **1.** Urediniospores with equatorial germ pores (× 800); **2.** A germinating teliospore (× 800); **3.** Teliospores with long pedicels (× 400).

namomeo-brunnea, poris germinationis 4, equatorialibus. Teliis hypophyllis, minutis, sparsis, subepidermalibus, erumpentis, pulvinatis, epidermide rupta conspicua, maculis 0.2–0.6 mm, diam., castaneo-brunnea; teliosporiis 30.5–50 × 20–28 μm, ellipsoideis, membrana apicalis incrassatus 3–5 μm, auranteo-brunnea, pedicello usque 140 μm longo, persistenti, teliosporae in situ germinativis.
Holotypus: Foliis vivis Gramineae, Kukatpally, Hyderabad (A.P.), India, August-September 1985, E. J. Ravinder, Herb. *B.*

Puccinia hyderabadensis Bagyanarayana & John Ravinder sp. nov. (figures 1–3).

Spermogonia and aecia not known. Uredinia hypophyllous, minute, scattered, subepidermal, erumpent, pulverulent, ruptured epidermis conspicuous, pale-brown, 0.2 mm diam.,; paraphysate, paraphyses hyaline, clavate to capitate; urediniospores 30–40 × 20–30 μm, ovate to ellipsoid, wall 2–3 μm thick, echinulate, cinnamon-brown, germ pores 4, equatorial, Telia minute, hypophyllous, scattered, subepidermal, erumpent, pulvinate, ruptured epidermis conspicuous, chestnut-brown, 0.2–0.6 mm diam; teliospores 30.5–50 × 20–28 μm, ellipsoid, wall apically thickened up to 3–5 μm, golden-brown, pedicels 140 μm long, persistent, teliospores germinating "in situ".
Holotype: On living leaves of a grass (Gramineae), Kukatpally, Hyderabad (A.P.), India, August-September 1985, E. J. Ravinder, Herb. *B.*

Puccinia hyderabadensis belongs to Group I of Cummins "Group system". There are many rust fungi in this group and some of them with which *P. hyderabadensis* can be compared are *P. sonorica* Cumm. & Hussain and *P. operta* Mundk. & Thirum. However, *P. hyderabadensis* differs from *P. sonorica* in having longer urediniospores with only 4 germ pores and longer teliospores. In addition *P. sonorica* is known from Mexico and USA only. The urediniospores of *P. operta* have 4–6 germ pores and the apical wall is thickened when compared to the uniformly thick urediniospores with only 4 germ pores in the case of *P. hyderabadensis*. Interestingly the teliospores of *P. hyderabadensis* show "in situ" germination which is not seen in the case of *P. sonorica* and *P. operta*.

The authors are grateful to Dr P. Ramachar, Department of Botany, Osmania University, who made a world monograph of the rusts parasitizing the grass tribe paniceae, for his kind help and to Dr Bharath Kumar for encouragement.

2 May 1987; Revised 15 July 1987

1. Cummins, G. B., *The rust fungi of cereals, grasses and bamboos*, Springer-Verlag, New York, 1971, p. 570.

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

K. MATHEN, J. J. SOLOMON, P. RAJAN and L. GEETHA
Central Plantation Crops Research Institute, Regional Station, Kayangulam, Krishnapuram 690 533, India.

Root (wilt) disease, the century-old debilitating malady of coconut, was suspected to be of virus origin for about three decades[1]. Based on experimental evidence[2-4] and observational data[2,5-7], 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 only[8] 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% glutarldehyde and paraformaldehyde[9] 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 citrate[10] 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 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 leaf[11]. Although MLO diseases are mostly transmitted by leafhoppers and plant hoppers and rarely by psyllids, there are instances in which members of Piesmidae, 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.

4 May 1987; Revised 15 June 1987

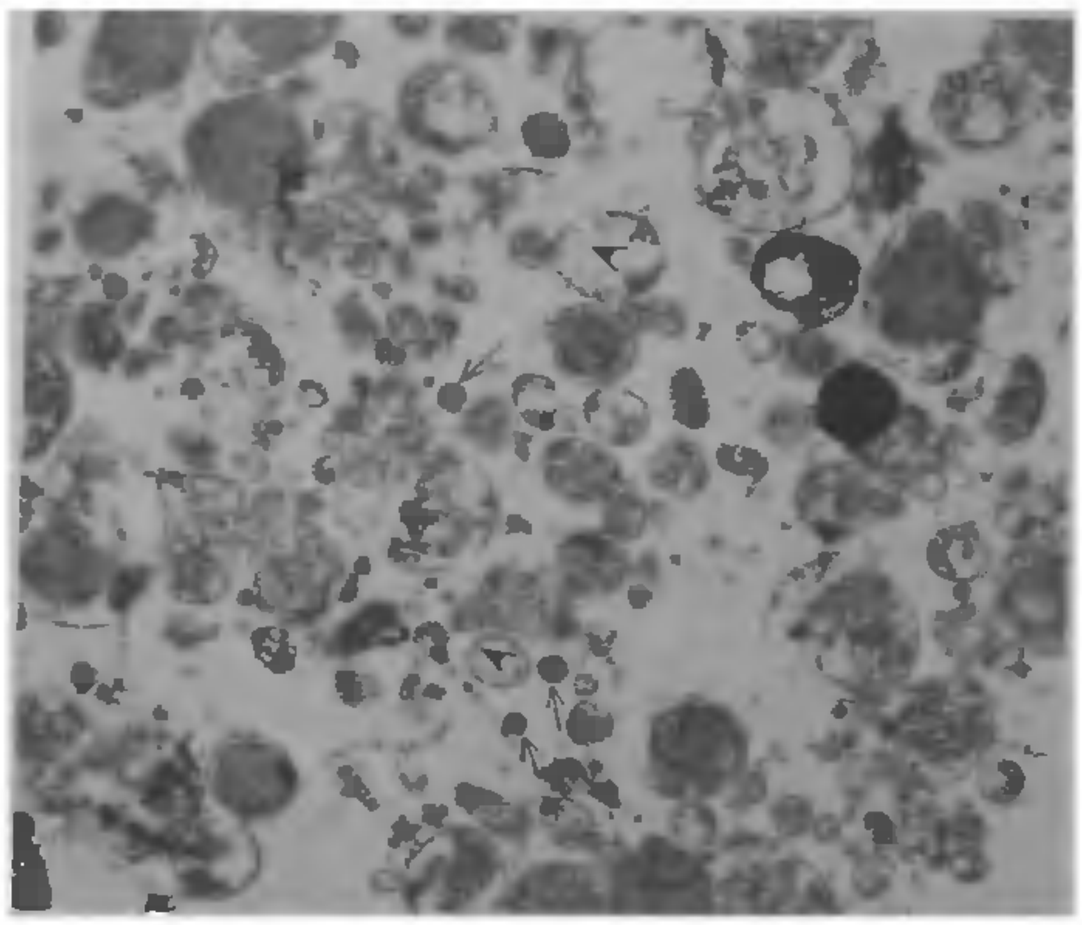

Figure 1. Mycoplasma–like organisms in salivery gland of lace bug: *Stephanitis typica* (arrows: elementary bodies; arrowheads: large forms).

1. Nagaraj. A. N., Davis, T. A. and Menon, K. P. V., *Indian Coconut J.*, 1954, **7**, 91.
2. Nagaraj, A. N. and Menon, K. P. V., *Indian Coconut J.*, 1956, **9**, 161.
3. Shanta, P., Menon, K. P. V and Patchupillai, K., *Indian Coconut J.*, 1960, **13**, 56.
4. Shanta, P., Thomas Joseph and Lal, S. B., *Indian Coconut J.*, 1964, **18**, 25.
5. Pillai, N. G., Sasikala, M. and Mathen, K., *J. Plantation Crops* 1980, **8**, 90.
6. Mathen, K., *Proc. All India Symposium on vectors and vector-borne diseases*, University of Kerala, Trivandrum 1982. p. 29.
7. Mathen, K., *J. Plantation Crops*, 1985, **13**, 56.
8. Solomon, J. J., Govindankutty, M. P. and Nienhaus, F., *Z. Pflanzenkr. Pflanzenschutz.*, 1983, **90**, 295.
9. Karnovsky, M. J., *J. Cell Biol.*, 1965, **27**, 137(abstr.)
10. Reynolds, E. S., *J. Cell Biol.*, 1963, **17**, 208.
11. Shanta, P. R. and Lakshmanan, A., *Curr. Sci.*, 1984, **53**, 265.

12. Harris. K. F. and Maramorosch, K., *Vectors of plant pathogens*, Academic Press, New York, 1980, p. 97.
13. Maramorosch, K. and Harris, K. F., *Leafhopper vectors and plant diseases*, Academic Press, New York, 1979, p. 220.

HISTOPATHOLOGY OF NUCLEAR POLYHEDROSIS WITH SIMULTANEOUS PARASITIZATION IN *CHRYSODEIXIS CHALCYTES* (ESP.) WITH *LITOMASTIX* SP.

R. J. RABINDRA and S. JAYARAJ
Centre for Plant Protection Studies, Tamil Nadu Agricultural University, Coimbatore 641 003, India.

A nuclear polyhedrosis virus (NPV) from *Chrysodeixis* (= *Plusia*) *chalcytes* (Esp.) has been reported earlier[1]. The encyrtid *Litomastix* sp. is an important parasite of *C. chalcytes* which is well established in New Zealand subsequent to its introduction from Australia[2]. This is a polyembryonic species and up to 1000 parasites may emerge from a single larva of *C. chalcytes*[3]. During microscopic examination of smears of NPV-infected larvae of *C. chalcytes*, it was found that several of them were found to carry the larvae of the parasitoid, *Litomastix* sp. This communication deals with the histopathology of nuclear polyhedrosis with simultaneous parasitization of *C. chalcytes* with *Litomastix* sp.

Fourth instar larvae of *C. chalcytes* collected from the weed *Flaveria australasica* (H.L.) were inoculated with the NPV by allowing them to feed on the leaves of *F. australasica* that had been previously dipped in the NPV suspension containing 10^7 polyhedral occlusion bodies (POB)/ml and dried in shade. Four days after infection when the larvae began to show signs of infection, they were killed in hot (60°C) alcoholic Bouin's fixative and bits of larvae fixed for 24 hr in cold alcoholic Bouin's fixative. Simultaneous microscopic examination of smears showed that several larvae had both the virus infection as well as the larvae of the parasitoid *Litomastix* sp. Larvae from control group without virus inoculation were also fixed on the fourth day. The fixed larval bits were washed in 70% alcohol repeatedly for several days until the yellow colour of the picric acid was removed, dehydrated in ethanol-butanol series and embedded in paraffin. Sections cut at 5μ were stained by the modified azan staining technique[4]. Photographs were taken with a Meopta (Czechoslovakia) microscope.

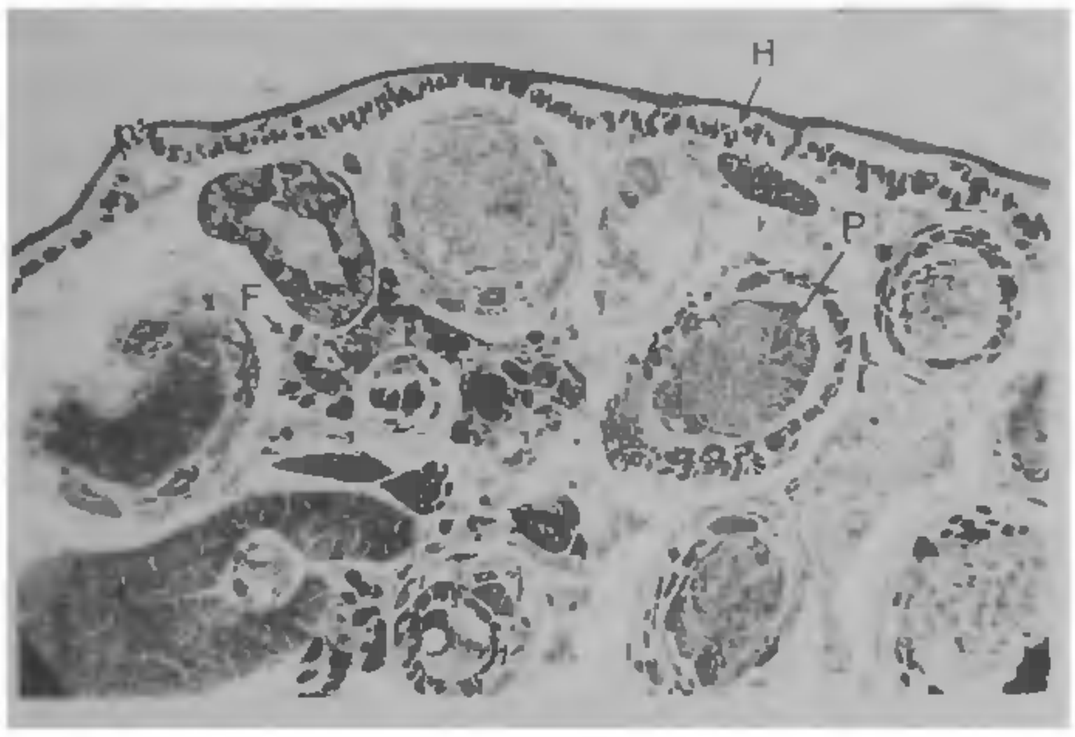

Figure 1. Cross-section of NPV infected larva of *Chrysodeixis chalcytes* showing the larvae of the parasitoid *Litomastix* sp. in the haemocoel. Note the polyhedra in the fat body (F) and hypodermis (H) of host as well as in the gut of the parasitoid (P). Line = 250 mμ.

In the cross-section of certain larvae of *C. chalcytes*, both NPV infection and the parasitoid *Litomastix* were seen (figure 1). POB could be seen in nuclei of hypodermis, fat bodies and trachea of *C. chalcytes*. Because of the parasitization with *Litomastix* sp. the fat body had completely been depleted. The gut of the parasitoids was full of POB. This indicated that the parasitoids had probably devoured the host tissues like the fat bodies after the virus development was completed in the nuclei of the cells. It is therefore evident that the parasitoids had been developing in the host unaffected by the virus infection. Compared with the parasitoids from uninfected larvae, there seemed to be no apparent difference in the anatomy of the parasitoids.

The development of the embryonic and larval stages of the internal gregarious parasite *Glyptapanteles* (= *Apanteles*) *militaris* (Walsh) was not affected by the fat body-infecting typical strain of NPV in the larvae of the armyworm *Pseudaletia unipuncta* (Haw)[5]. Development was however adversely affected by the hypertrophy strain of the NPV. A haemolymph factor from the armyworm infected with the hypertrophy strain of NPV was found to be toxic to *Apanteles militaris* (Walsh)[6]. In the case of the parasites of *Lymantria dispar* (L.), the parasite development was affected only if the larvae died of the virus before the parasite development was completed[7].

In the present studies, normal emergence of *Litomastix* sp. was observed in certain NPV-inoculated larvae suggesting that the NPV infection had not affected the parasite development. These find-