STUBBORN, GREENING, and RELATED DISEASES

Visualization of *Spiroplasma Citri* in the Leafhopper *Scaphytopius Nitridus* (De Long)

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Spiroplasma citri Saglio *et. al.* is the causal agent of stubborn disease of citrus (Markham *et al.*, 1974). Unlike most other phytopathogenic mycoplasmalike organisms (PMLO), *S. citri* can be cultured on artificial media (Fudl-Allah *et al.*, 1971; Saglio *et al.*, 1971). Most PMLO are known to be vectored by one or more leafhoppers or psyllids (Whitcomb and Davis, 1970; Kaloostian *et al.*, 1971), but the natural vector or vectors of stubborn have been difficult to discover. Recently workers in England (Daniels *et al.*, 1973; Markham *et al.*, 1974) obtained transmission by injecting *S. citri* cultures into *Euscelis plebejus* (Fallen), and feeding the injected insects on citrus. In California, *S. citri* was cultured from macerates of the beet leafhopper, *Circulifer tenellus* (Baker), collected in citrus environs (Lee *et al.*, 1973). Likewise, greenhouse-reared healthy *C. tenellus* (G.N. Oldfield, personal communication) and *Scaphytopius nitridus* (De Long) leafhoppers acquired the organism from experimentally infected citrus plants (Kaloostian *et al.*, 1975). Transmission of *S. citri* to healthy citrus occurred at a very low rate.

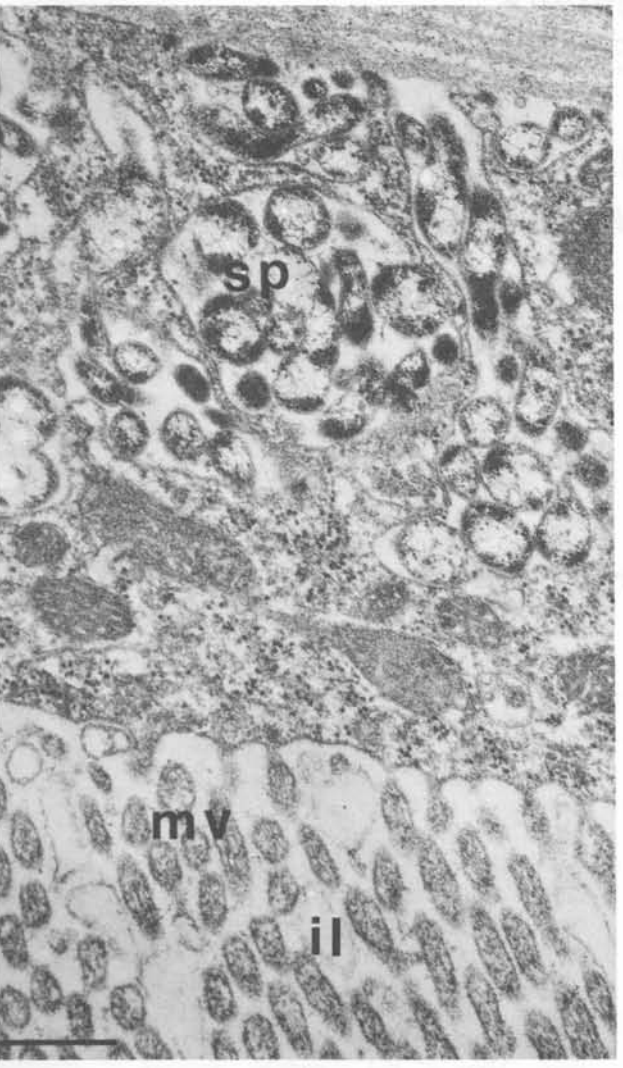
In another approach to the problem of acquisition of the PMLO, we fed greenhouse-reared healthy insects a concentrated suspension of *S. citri* through a Parafilm membrane. The leafhoppers readily acquired the PMLO as evidenced by its reisolation from the leafhopper macerates. In a very few cases transmission to citrus was also effected (Rana *et al.*, 1975).

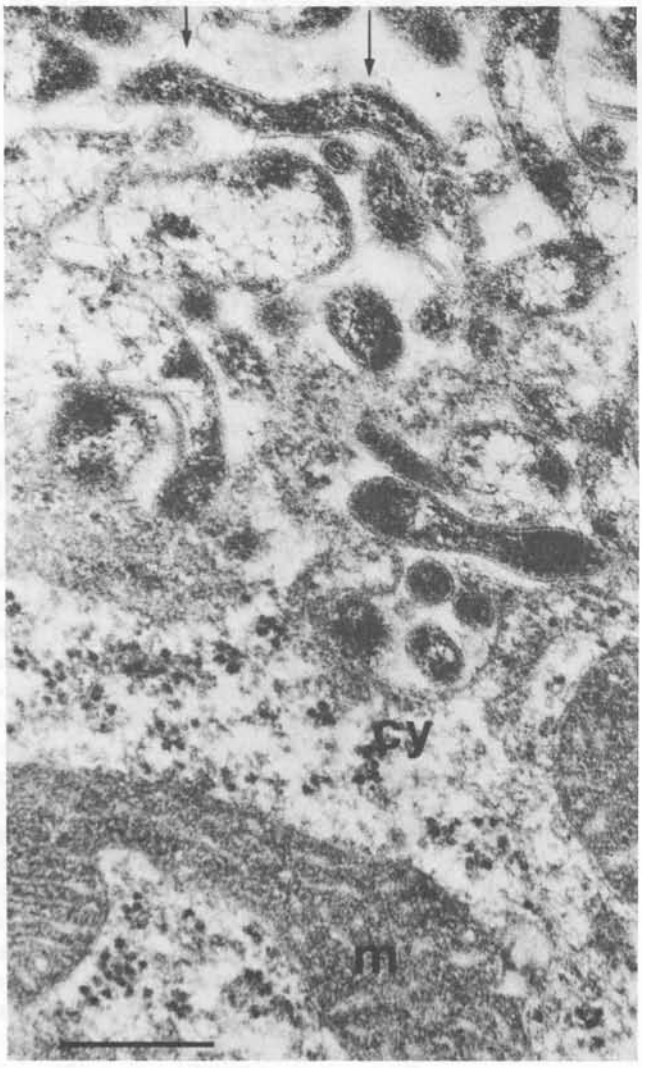
Although these experiments indicated that *S. citri* multiplies within leafhoppers, they provided no visual evidence that it was present inside the insect cells. For this investigation we fed *S. nitridus* adults on 5 per cent sucrose solutions containing *S. citri.* (Groups of these insects were macerated and *S. citri* was isolated from most groups. After 40 days several individuals were dissected, fixed and embedded for electron microscopy.

Mycoplasmalike organisms (MLO) were found abundantly in thin sections of some, but not all leafhoppers. MLO were present in several organs of the insect, namely, intestine (figs. 1 and 2), salivary glands (fig. 3), and intact (fig. 4A,B) or degenerating somatic muscles. In the latter, groups of MLO were encased in sack-like membranous structures (fig. 5). Each MLO contained ribosomes and a central fibrillar nuclear area and was surrounded by a unit membrane 7.5 nm thick. Most of the organisms appeared round or spherical, but this could be attributed to cross-sections of the elongated or sausage-shaped bodies frequently encountered. Occasional bodies with a spiral morphology were also seen (figs. 2 and 5).

Incidentally, in several insects unidentified prokaryotes with a well defined cell wall were found extracellularly in the intestinal lumen and, on occasion, within cells of the head region.

We believe that the observed MLOs are actually *S. citri* for several reasons. They were never found in leafhoppers not fed on the *S. citri* suspensions, but were





Figs. 1 and 2. *Spiroplasma citri* bodies (sp) in intestinal epithelial cells of *Scaphytopius nitridus*. Arrows indicate a body with spiral morphology; m = mitochondria; cy = ground cytoplasm; il = intestinal lumen; mv = microvilli. Bar is 500 nm.

present in many insects that had been so fed. *S. citri* had been consistently isolated from other individuals in the same group as those sampled for microscopy. Finally, all insects were from a colony reared for many generations on healthy plants in cages in a controlled environment. They harbored no known pathogens.

The scarcity of *S. citri* bodies with distinctive spiral morphology may be attributable to the "growth media" present within the insect host, the stage of the organism's life cycle (Fudl-Allah and Calavan, 1974), and the fixation method (Lemcke, 1972).

On the basis of present evidence, we conclude that *S. citri* invades the tissues of the insect in a manner very similar to other PMLO that multiply in both plant and vector hosts.

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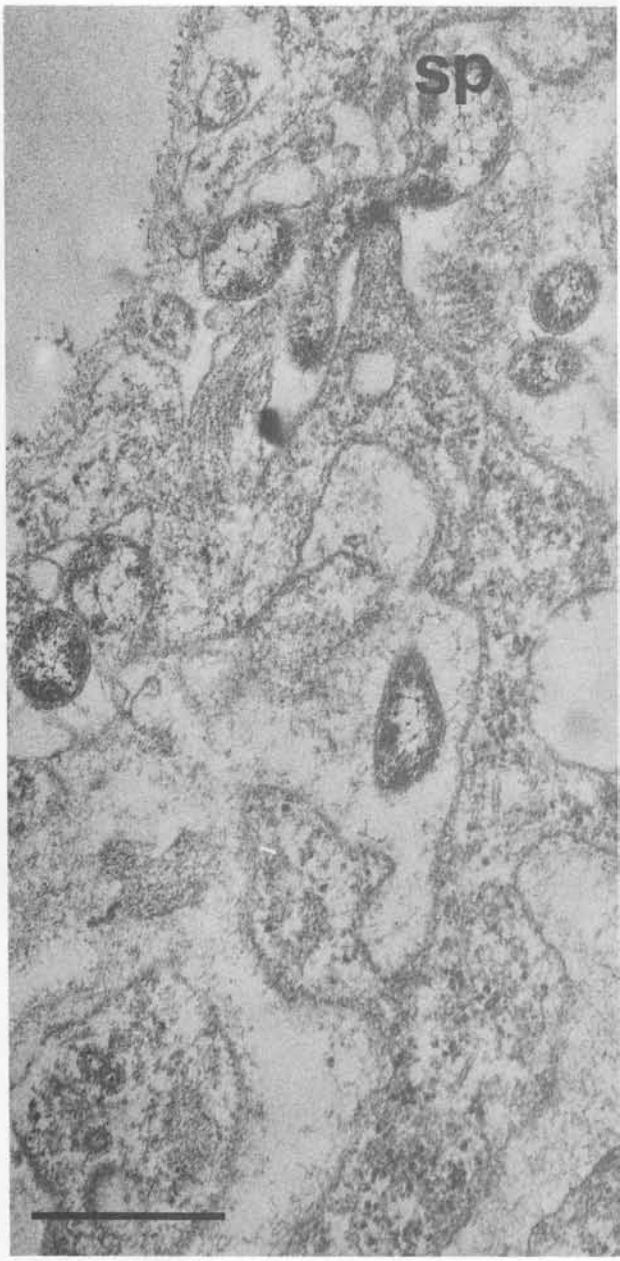

Fig. 3. *Spiroplasma citri* cells (sp) inside salivary glands of *Scaphytopius nitridus*. Bar is 500 nm.

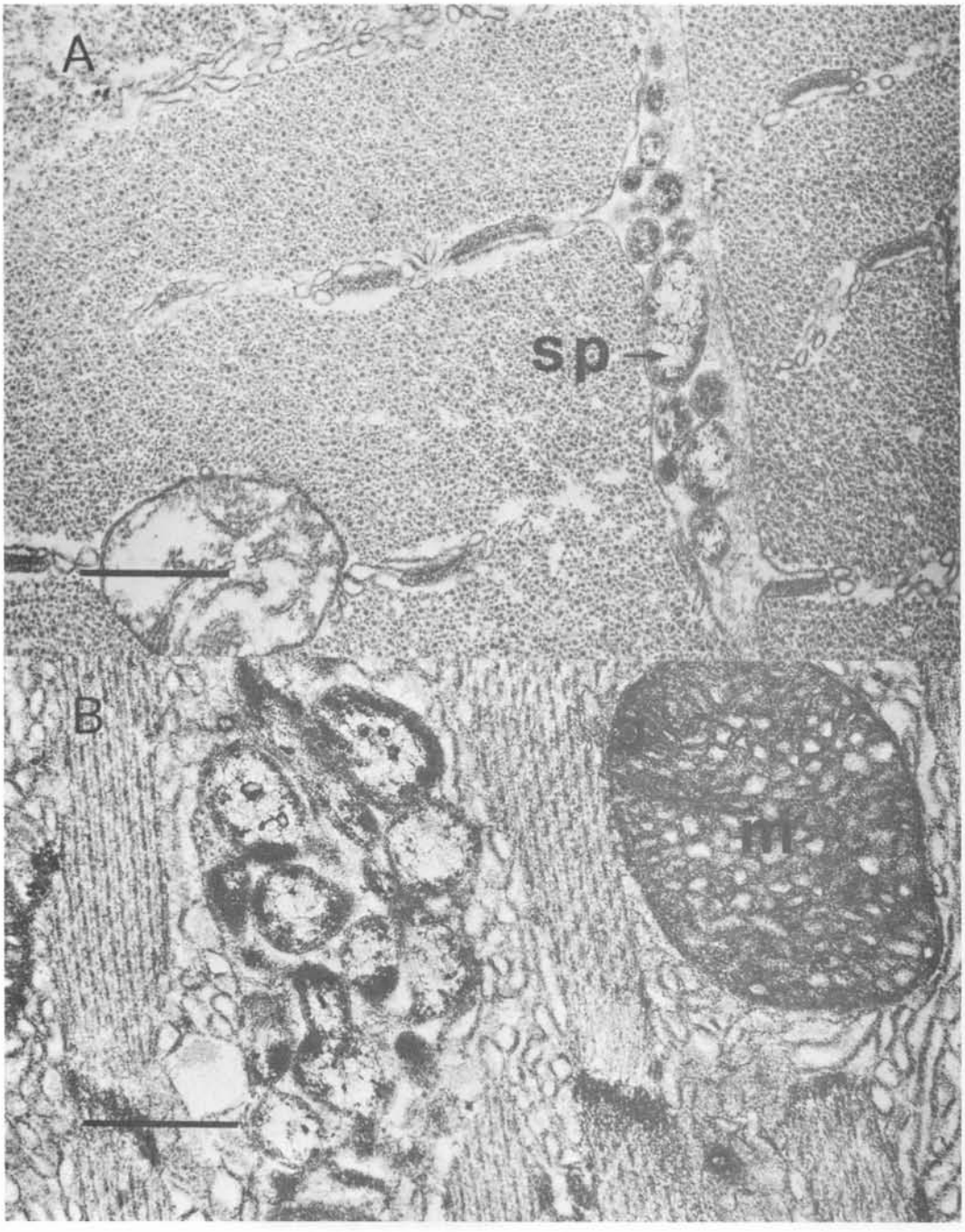

Fig. 4. *Spiroplasma citri* cells (sp) in muscular tissue of the leafhopper. m = mitochondria. Bar is 500 nm.

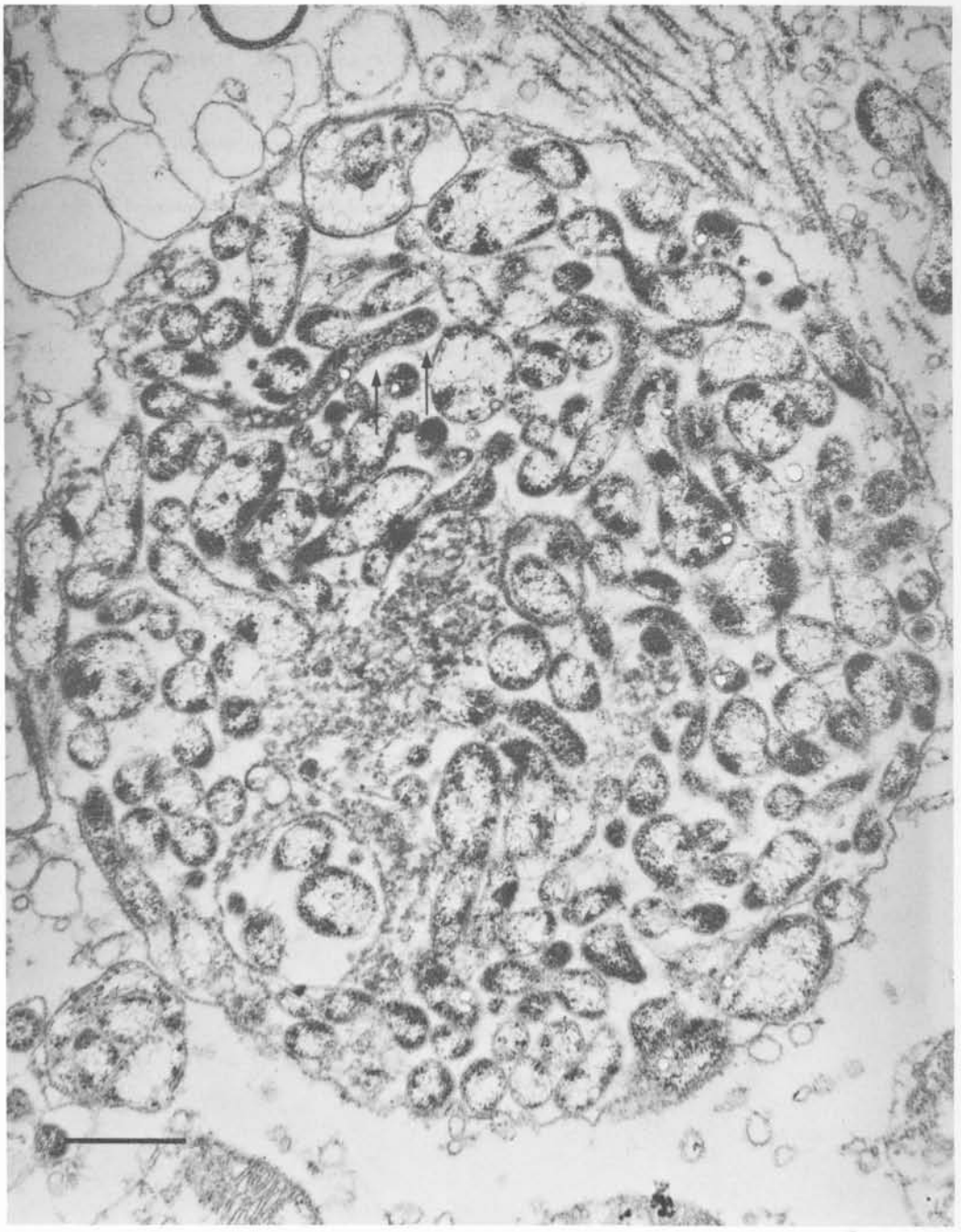

Fig. 5. *Spiroplasma citri* cells enclosed in a membranous sack-like body in degenerated muscular tissue of *Scaphytopius nitridus*. Arrows point to a cell with spiral morphology.

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