

## PHYTOPATHOLOGICAL NOTES

### Occurrence of Mycoplasma-like Bodies in Phloem of Stubborn-Infected Citrus Seedlings

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#### ABSTRACT

Pleomorphic, sometimes filamentous, mycoplasma-like bodies, 50-500  $\mu$  diam, with a trilaminar unit membrane, were found in phloem of leaves from stubborn-infected but not in phloem of healthy, sweet orange seedlings. We suggest that stubborn of citrus may be caused by a mycoplasma-like pathogen rather than by a virus. *Phytopathology* 60:1525-1526.

Stubborn disease causes general stunting, excessive proliferation of axillary buds, small cupped mottled leaves, and seed abortion in severely affected plants of various *Citrus* spp. (2). The disease is transmissible by grafts, and possibly by a vector. Since no fungus or bacterium has been implicated as its causal agent, stubborn has generally been called a virus disease. But, attempts to transmit the presumed causal virus mechanically and to isolate, purify, and photograph it under the electron microscope have failed.

Yellows-type plant diseases hitherto ascribed to viruses may be caused by mycoplasma-like bodies. This suggestion is based on the occurrence of such bodies in phloem cells of affected plants and the effectiveness of tetracycline antibiotics in suppressing disease symptoms (1, 3, 4, 5, 7, 9). In our unpublished preliminary studies, tetracycline antibiotics, chlortetracycline hydrochloride and tetracycline hydrochloride, but not penicillin G, consistently suppressed symptoms of stubborn in citrus plants growing in culture solution. This fact and the similarity between symptoms of stubborn and those of other yellows-type diseases in which mycoplasma-like bodies have been detected prompted us to examine tissues from stubborn-infected plants with the electron microscope.

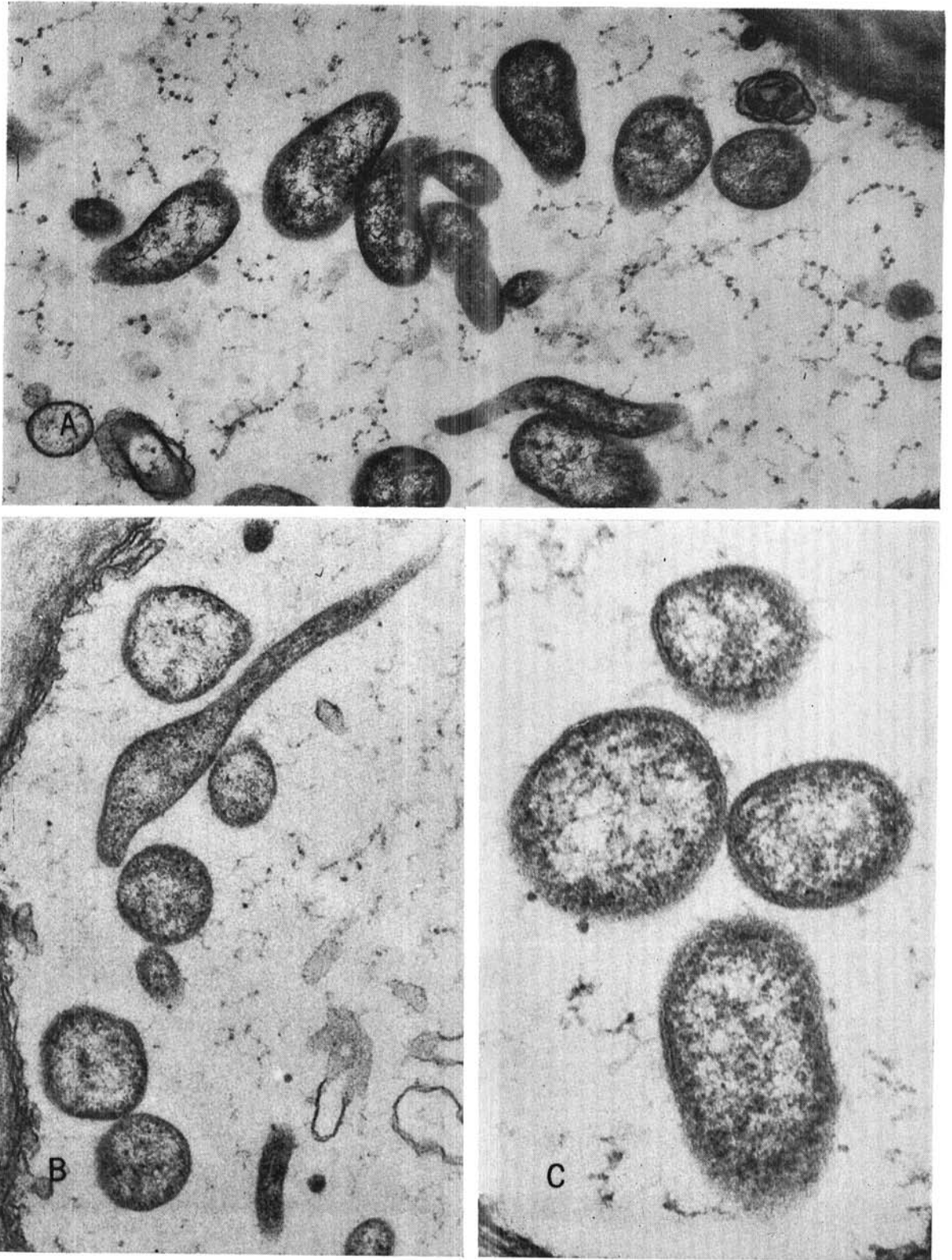
Greenhouse-grown Madam Vinous sweet orange seedlings experimentally infected with severe stubborn disease and free of other known graft-transmissible pathogens were used for this study. Pieces of tissue  $1 \times 1$  mm were removed from the midvein of fully expanded young leaves showing stubborn symptoms. Samples were fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer, pH 7.4, for 3 hr at 4 C, rinsed, and postfixed in 2% (w/v) osmium tetroxide in the same buffer for 3 hr at 4 C. After dehydration through graded acetone series, samples were passed through two final changes in propylene oxide and then embedded in Epon mixture (6). Thin sections were cut with glass knives

on a Porter-Blum MT-2 ultramicrotome. Sections were collected with Formvar-coated grids, doubly stained in a saturated solution of uranyl acetate for 1 hr and lead citrate for 10 min (8), and examined with an RCA EMU-3B electron microscope.

No virus particles were seen, but numerous pleomorphic bodies were present in some of the mature sieve-tubes of infected plants (Fig. 1-A, B). Such bodies were absent in comparable tissue from leaves of healthy seedlings. These bodies were from 50 to 500  $\mu$  in size, and the larger bodies contained ribosome-like granules and fibrils not apparent in the smaller more electron dense bodies. A triple-layered unit membrane was apparent in some sections (Fig. 1-C). In size and morphology, these bodies are similar to mycoplasma-like structures reported by other workers to occur in phloem cells of plants affected by yellows-type diseases. Our results indicate that these mycoplasma-like organisms may be the causal agent of stubborn disease. Proof of the nature of the pathogen awaits the completion of Koch's postulates. While this manuscript was in preparation, D. Lafèche and J. M. Bové of Versailles, France (*personal communication*), informed us that they found mycoplasma-like bodies in phloem cells of greenhouse-grown sweet orange seedlings inoculated with the Nelspruit strain of greening disease of citrus.

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**Fig. 1.** Mycoplasma-like bodies in cross sections through sieve tubes of stubborn-infected sweet orange leaf. **A)** Portion of sieve tube showing several mycoplasma-like bodies ( $\times 42,000$ ). **B)** Spherical and filamentous mycoplasma-like bodies containing ribosome-like granules and fibrils ( $\times 49,000$ ). **C)** Mycoplasma-like bodies with a unit membrane surrounding each body ( $\times 115,000$ ).