# Possible Replicative Forms of a Mycoplasmalike Organism and their Location in Aster Yellows Diseased Nicotiana and Aster

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ABSTRACT

Mycoplasmalike bodies were observed in phloem cells of tobacco (*Nicotiana rustica*) and aster (*Callistephus chinensis*) plants suffering from aster yellows. Most cells containing the mycoplasmalike bodies were sieve elements devoid of recognizable plant organelles, but bodies were occasionally seen in elements containing apparently intact mitochondria, plastids, or degenerate nuclei. The bodies were rarely observed in parenchyma cells adjacent to mature sieve elements. Pleomorphism was evidenced by a wide variety of sizes, shapes, electron densities, and arrangements. The bodies were bounded by a clearly defined membrane with no evidence of a second membrane or cell wall. Single spherical bodies varied in diam from 75 to 600 mμ; the long dimension of the elongated forms attained lengths of 1 μ. Structures suggesting chains, binary fission, budding, and filamentous growth may represent stages in the life cycle of the organism in its plant host. Phytopathology 60:284-292.

The long-held assumption of a viral etiology of aster yellows has recently been questioned (1, 3, 8, 13). On the basis of electron microscopy and chemotherapy with antibiotics, these reports suggest a mycoplasmalike organism as the probable causal agent. Although mycoplasma species are now regarded as potentially significant pathogens of man, animals, and insects as well as plants, the reproductive cycle of members of this group of organisms is still poorly known (9). Although many reports are available concerning in vitro studies of mycoplasma, relatively few studies are available on its morphology in vivo. Also, the distribution of mycoplasmalike bodies in yellows-diseased plant tissues has not been established. This report presents evidence that mycoplasmalike bodies are limited to phloem sieve elements and adjacent parenchyma cells in both aster and tobacco infected with the aster yellows agent. The findings also provide evidence for stages in the reproductive cycle in vivo for this organism which include formation of filaments and chains of bodies. A preliminary report on a part of this work has been presented (16).

METHODS.—Greenhouse-grown plants of tobacco (*Nicotiana rustica* L.) and aster (*Callistephus chinensis* [L.] Nees.) were inoculated with aster yellows agent via the leafhopper vector *Macrosteles fascifrons* (Stål.). Two types of leaf material were harvested. In one case, material exhibiting different degrees of symptom development was used. Young, expanding symptomless leaves from chronically infected plants comprised one sample; young leaves with vein-clearing and slight chlorosis, and older leaves with severe chlorosis, made up two other samples. In the other case, leaves of comparable ages were taken from plants 7, 17, 27, or 37 days after exposure to inoculative insects regardless of symptom expression in the leaves. Symptoms in such plants first appeared 10 days after inoculation. Leaf pieces 1-2 $mm^2$ and containing either small, medium-sized, or large veins were fixed in 3% glutaraldehyde in 0.15 M phosphate buffer pH 7, postfixed in 2% osmium tetroxide in the same buffer, dehydrated in an ethanol series, and embedded in araldite resin. Sections were stained with uranyl acetate and lead citrate.

RESULTS.—Mycoplasmalike bodies were limited to the phloem of leaf samples from aster and tobacco plants that showed aster yellows symptoms. Bodies were not observed in any type of xylem cell nor in mesophyll cells. Mainly, they were observed in sieve elements devoid of recognizable organelles or vacuole. The pleomorphic bodies were occasionally present in elements containing apparently intact mitochondria, dictyosomes, endoplasmic reticulum, or a degenerate nucleus. Not all sieve cells in a given vascular bundle contained bodies; and, within a given square mm of leaf, some small veins contained bodies and some did not. Elements adjacent to those infected appeared unaffected anatomically. The mycoplasmalike bodies were rarely observed in phloem parenchyma cells adjacent to sieve elements.

In sieve elements, the bodies were mostly dispersed in the cytoplasm, and individual organisms were intermixed with strands of slime. The numbers of bodies within a section of a cell varied from one to hundreds. Often they appeared as various sized "packages" bordered by the host cell plasmalemma (Fig. 1). In the parenchyma cells, mycoplasmalike bodies were also present in the cytoplasm, but here they formed a compact, electron-dense mass (Fig. 5). The bodies were not found in the vacuole, but occasionally large crystals were present in vacuoles of parenchyma cells containing the bodies or in parenchyma cells adjacent to sieve elements containing bodies.

A wide variety of forms and sizes of bodies was observed (Fig. 1, 2). Single spherical bodies varied in diam from about 75 to 600 mμ. The elongated forms were of two types. The irregularly shaped elongated bodies observed in sieve elements may be degradative forms. They are not uniform in electron density, nor are they uniform in width (Fig. 2). The regular-shaped elongated bodies in the parenchyma cells, because of

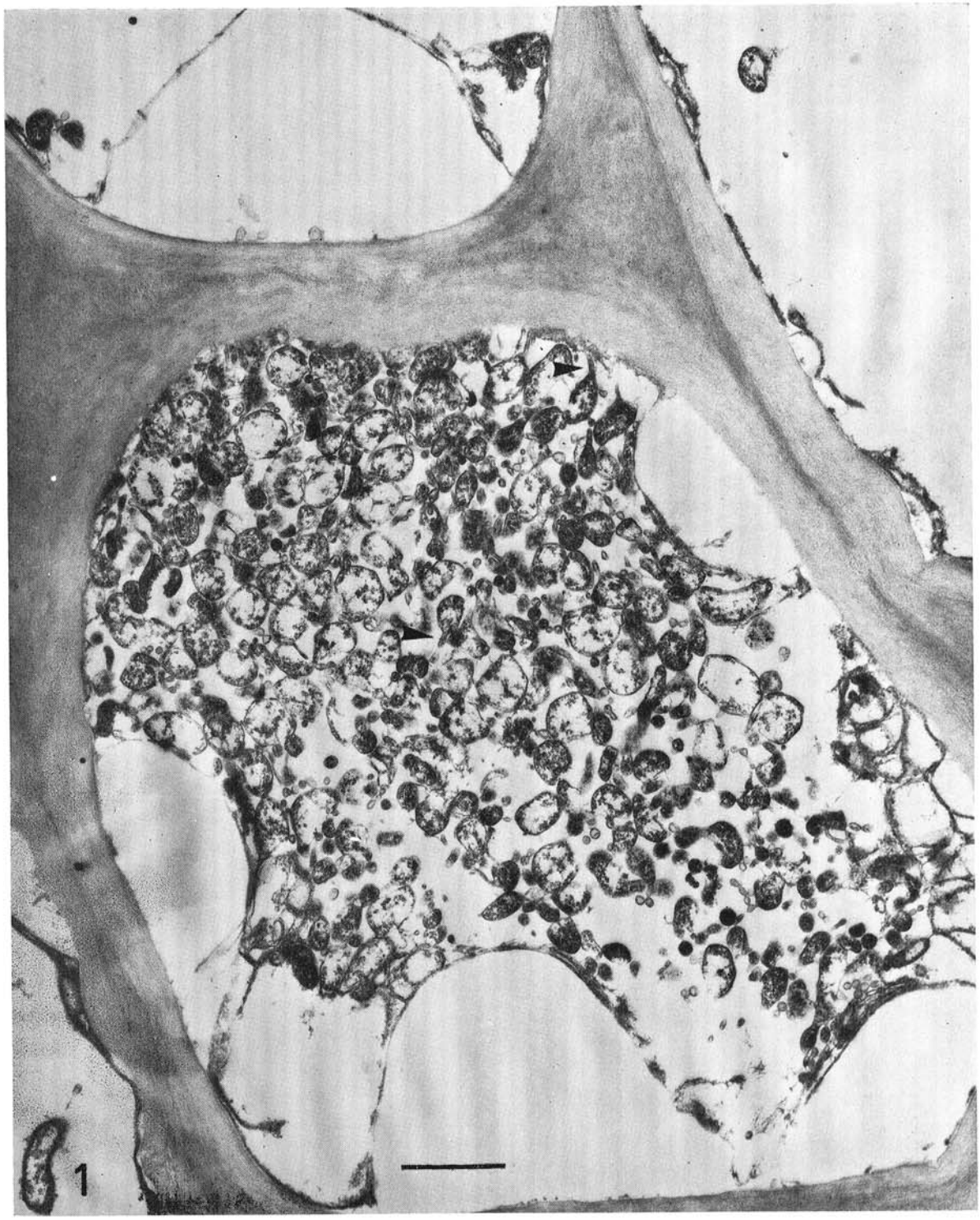


**Fig. 1.** Section of phloem sieve element from symptomless leaf of aster yellows diseased tobacco plant showing variety of mycoplasmalike forms enclosed within the plasmalemma. Filamentous growth (arrows) is associated with several of the larger spherical organisms. Bar equals 1 μ.

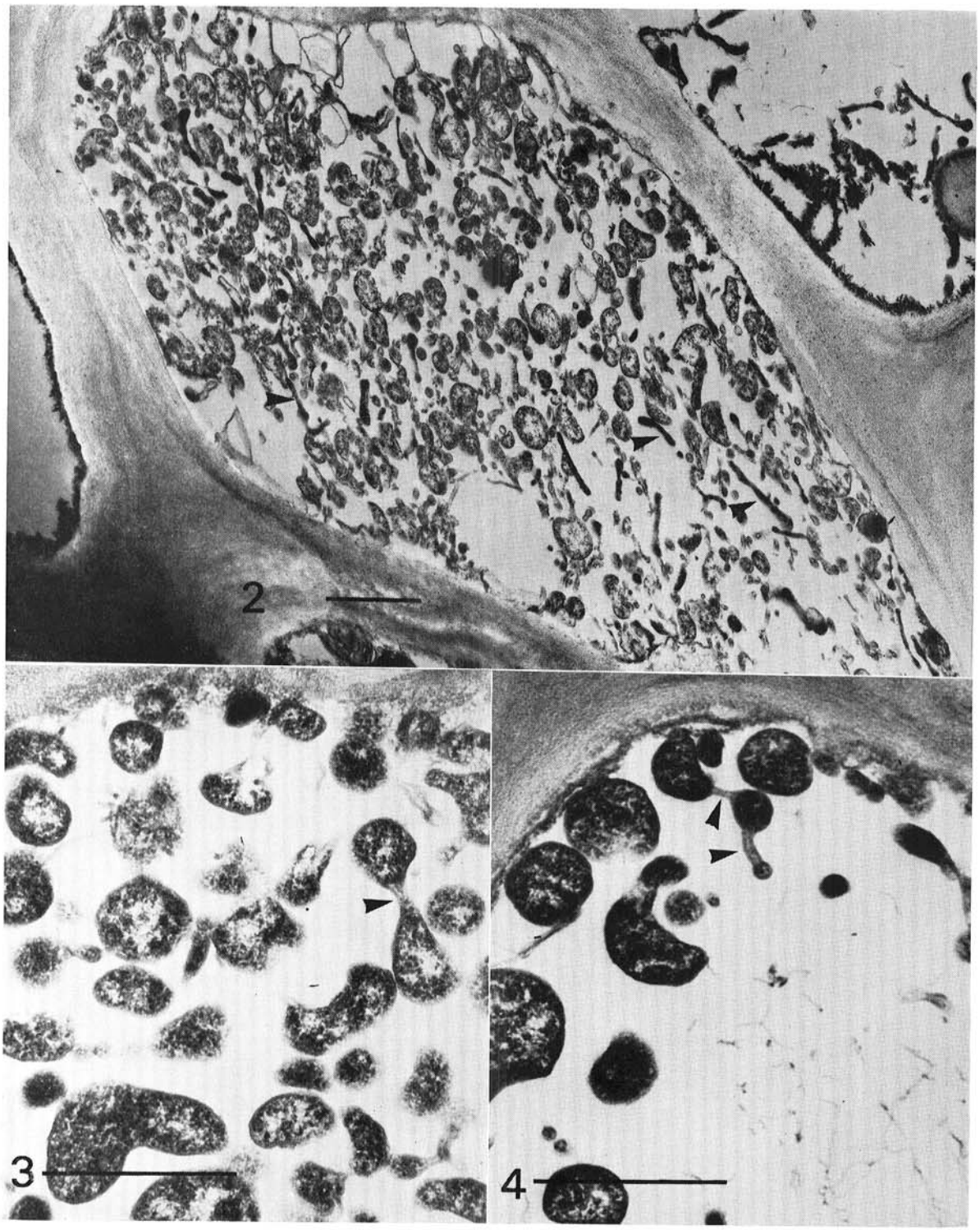


**Fig. 2-4.** **2)** Section of sieve element from chlorotic leaf of aster yellows diseased tobacco plant showing presence of irregular elongated forms (arrows) intermixed with other pleomorphic forms. **3)** Structure indicative of binary fission of a mycoplasmalike organism. **4)** Structure resembling filamentous growth of mycoplasmalike organism. Spherical bodies appear connected by thin filaments (arrows). Bars equal 1 μ.

their contents and appearance, are interpreted to be viable organisms. These are about 100 mμ in width, about 1 μ in length, and more uniform in electron density. The small spherical bodies (75 to 100 mμ) were also of two types. Some were devoid of subcellular components; some contained ribosomes and electron-dense material. Both types were often found in the same cell (Fig. 2). The apparently empty bodies may be vesicular products formed during breakdown of larger bodies. The smaller spherical forms with recognizable contents are presumed to be viable forms capable of growth into the larger spheres. Both types of small bodies often were found in the form of chains (Fig. 1, 2).

The larger spherical forms (300 to 400 mμ) were occasionally seen in a chainlike configuration in aster (Fig. 7), but in tobacco this configuration was rare. Also, the larger spheres sometimes formed filaments (Fig. 2) and buds (Fig. 8). A structure suggesting binary fission of the larger spheres is shown in Fig. 3. There was a conspicuous lack of structures resembling inclusion bodies.

Ultrastructurally, the bodies were bounded by a clearly defined trilaminar membrane, and contained

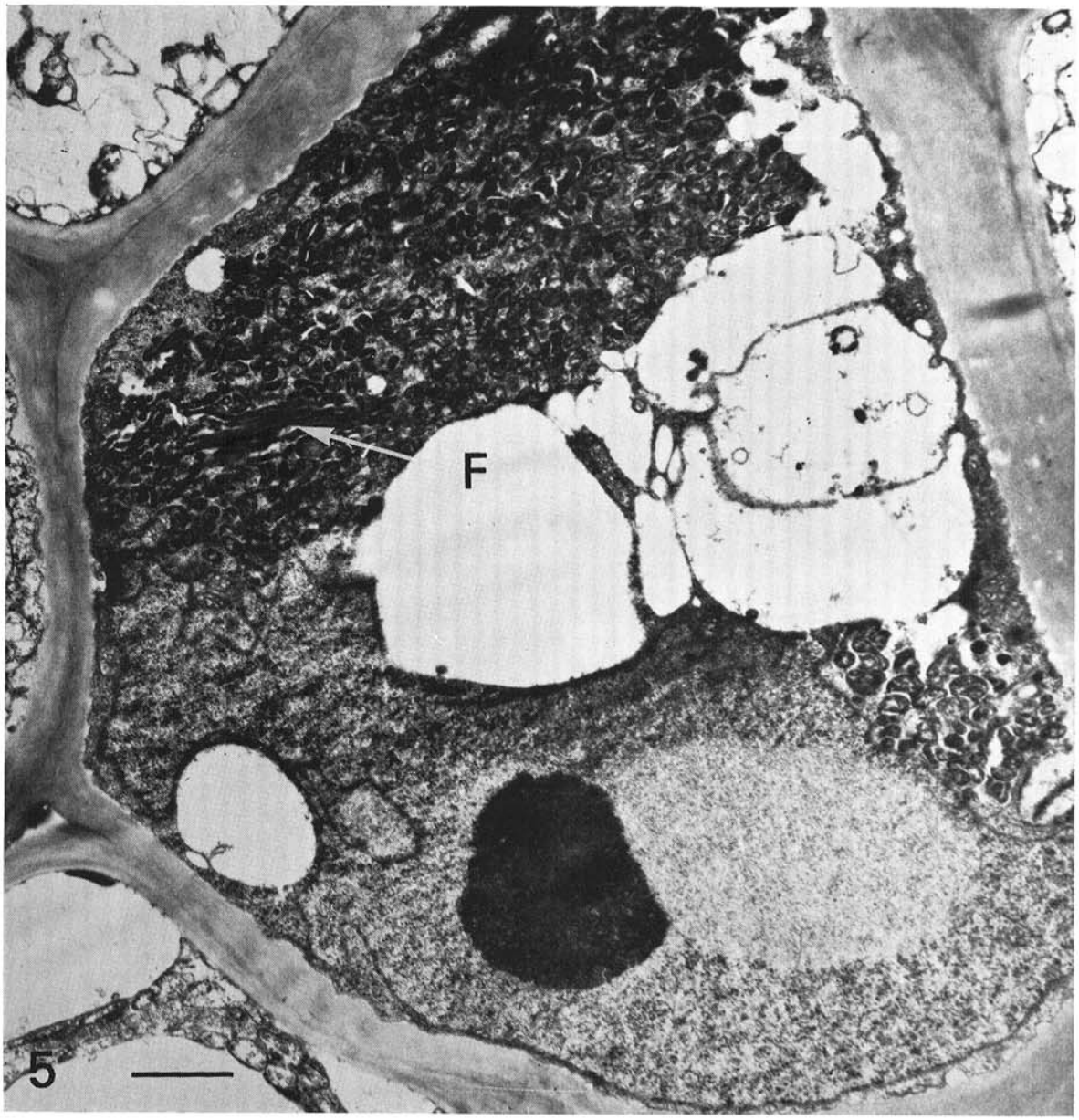


**Fig. 5.** Phloem parenchyma cell of aster yellows diseased tobacco plant containing dense mass of mycoplasmalike bodies in the cytoplasm. Mass includes several uniform elongated forms. Bar equals 1 μ.

ribosomes and fine strands presumed to be nucleic acid (Fig. 8). In the closely packed array of bodies in parenchyma cells a distinct electron transparent border was occasionally observed (Fig. 6). This border may have been a fixation artifact.

Structures such as that shown in Fig. 4 appeared to be made up of spherical bodies joined by thin filaments. Possibly, one body produced a filament at the end of which another spherical body was formed. The second body, in turn, may have initiated another cycle of filament-spherical body formation. The structure shown in Fig. 4 appeared to have a partly formed body at the end of the second filament. The filaments in this structure were about the same width as the chains of smaller spherical bodies (Fig. 1, 2).

There was an obvious difference in the relative numbers of various forms present in symptomless and in severely chlorotic tissues of a given plant. A greater proportion of electron-dense, irregularly elongated forms was present in areas with severe symptoms. Concomitantly, the spherical forms, both large and small, were relatively fewer in number in the severely chlorotic areas. Essentially the same pattern was observed in a timed study. In the earliest collection, 7 days after diseased insects fed on plants and 3 days before the first appearance of symptoms, no mycoplasmalike bodies were observed. At 17 days, spherical bodies predominated, but in the later collections, there was a greater proportion of the irregular elongated forms.

In an attempt to find mycoplasmalike bodies in inoculated plants before the appearance of symptoms, leaf material from three plants was collected 7 days after inoculation. About 27 $mm^2$ of leaf areas comprising, small, medium, large, and main veins were sectioned and observed. No mycoplasmalike bodies were positively identified in any cell.

No mycoplasmalike bodies were observed in sections of noninfected control plants. In each experiment, like numbers of leaves from both aster and tobacco similar in age to those harvested from infected plants were fixed, embedded, sectioned, and observed.

DISCUSSION.—As in previous reports (1, 3, 8, 13), the evidence implicates a mycoplasmalike organism in the etiology of aster yellows. The bodies associated with diseased plants are morphologically similar to known mycoplasmas. The presence of the organism in diseased plants and its absence in noninfected plants, while not conclusive proof, indicate a direct relationship with disease. Its presence in sieve elements is consistent with the long-held assumption of phloem limitation of the aster yellows agent based on feeding by the vector, and is consistent with requirements for aster yellows transmission. The limited distribution among various veins within the same portion of a young leaf from a chronically infected plant is not readily explained.

Differences in morphology of the organism in sieve elements as compared with that of bodies in parenchyma cells might be due to differences in the cellular environments in the two cell types. Mature sieve elements, while they are living cells, are devoid of a nucleus and ribosomes, and may thus represent an environment between a normal nucleated cell and a nonliving cell (4). Morphology of mycoplasmas was reported to be dependent on the fatty acid composition of the growth medium (12), and may differ in broth or agar culture (2).

The electron transparent border (Fig. 6) may be caused by shrinkage of the organism during processing for electron microscopy, or may be due to the presence of a matrix as reported by Gourlay & Thrower (7). The formation of filaments and budding and the presence of chains of small empty bodies described were also observed in infected plants by Maillet et al. (11).

Translocation or cell-to-cell movement of the mycoplasmalike bodies may involve elongated forms as proposed by Shikata & Maramorosch (14), or perhaps filamentous forms (Fig. 4). Sieve pores in mature sieve elements are large enough to accommodate the elongated form; however, filaments or small spherical bodies might, because of their smaller size, be moved more readily, or they might penetrate adjacent cells more easily. Either pliability of the organism, due to its lack of a rigid cell wall, or growth of filaments could account for apparent cell-to-cell movement of the larger bodies. Van Boven et al. (15) found that bodies of streptococcal L-forms unable to pass a certain filter were able to grow through this same filter when it was placed on a medium suitable for growth.

The interpretation of morphology and reproduction of mycoplasmas is in a state of confusion and disagreement (5). Hayflick & Chanock (9) present a good review of the two major schools of thought on the growth patterns of this group of organisms. Characteristic of one group is the pattern described by Freundt (5). According to his scheme, an elementary body extrudes one or more exceedingly thin filaments that terminate in a tiny body similar in size to the elementary body. The next stage of growth is chain formation initiated by appearance of bodies within the filaments. The chains separate into new elementary bodies. Freundt's scheme is based on phase contrast observation of in vitro cultures. The second group of workers holds that the filaments are atypical, and that the reproductive cycle consists of formation of inclusions within large spherical bodies. In this second type of cycle, a small body, termed a minimal reproductive unit, enlarges into pleomorphic bodies which form, within them, inclusions that eventually give rise to minimal reproductive units. Interpretations of the second group are also based on in vitro cultures. Apparently, much of the controversy between the two groups of workers stems from differences in cultural conditions. More recently it was reported that under certain cultural conditions reproduction of *Mycoplasma pneumoniae* was by binary fission (6).

If all the various forms illustrated in this study are from one organism, the results suggest the importance of filaments and chains of bodies in reproduction, rather than a cycle involving inclusion bodies. Hirumi & Maramorosch (10) reported inclusions in myco-

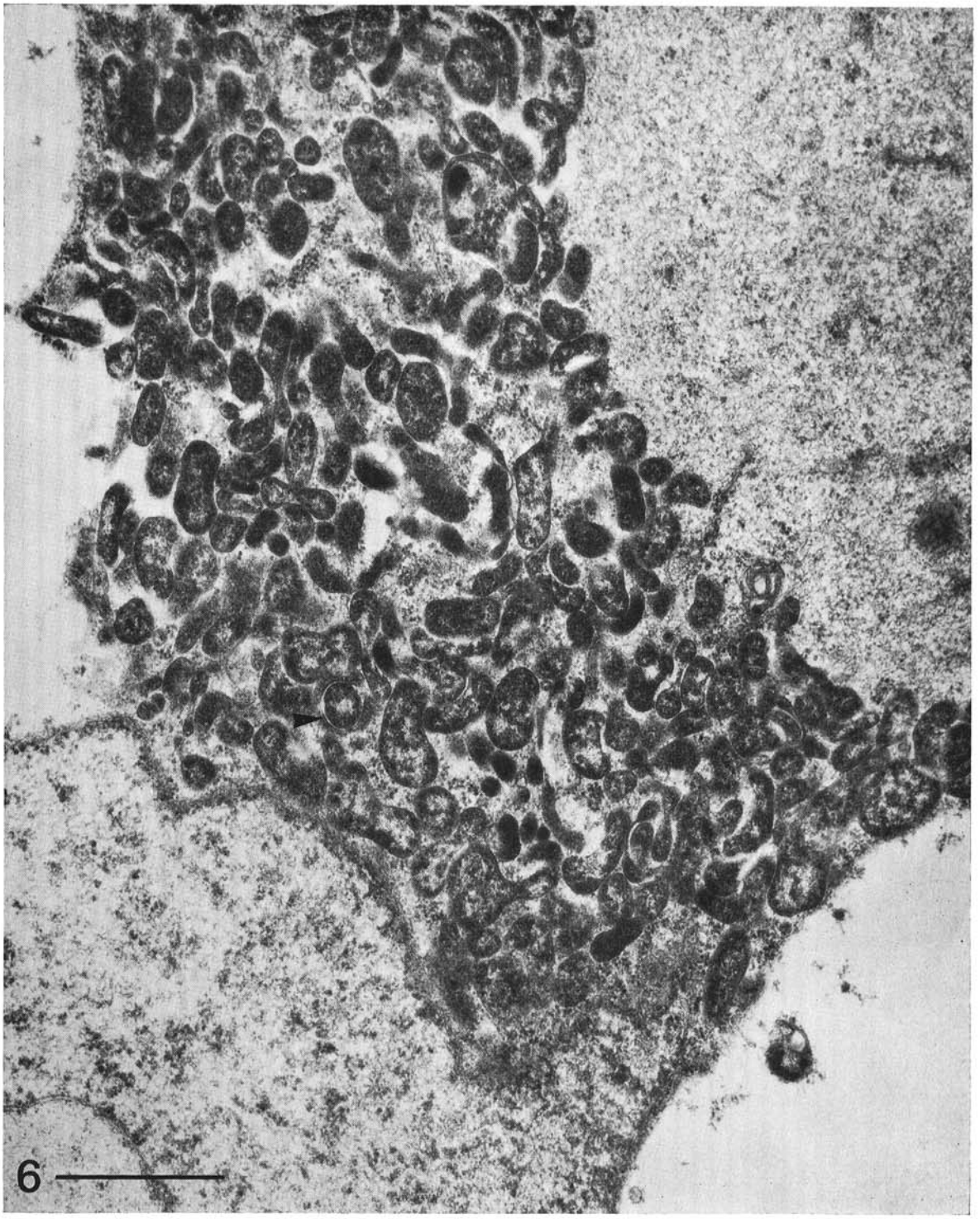


**Fig. 6.** Packet of mycoplasmalike bodies in cytoplasm of infected phloem parenchyma cell. Distinct electron transparent border is apparent around some bodies. Bar equals 1 μ.

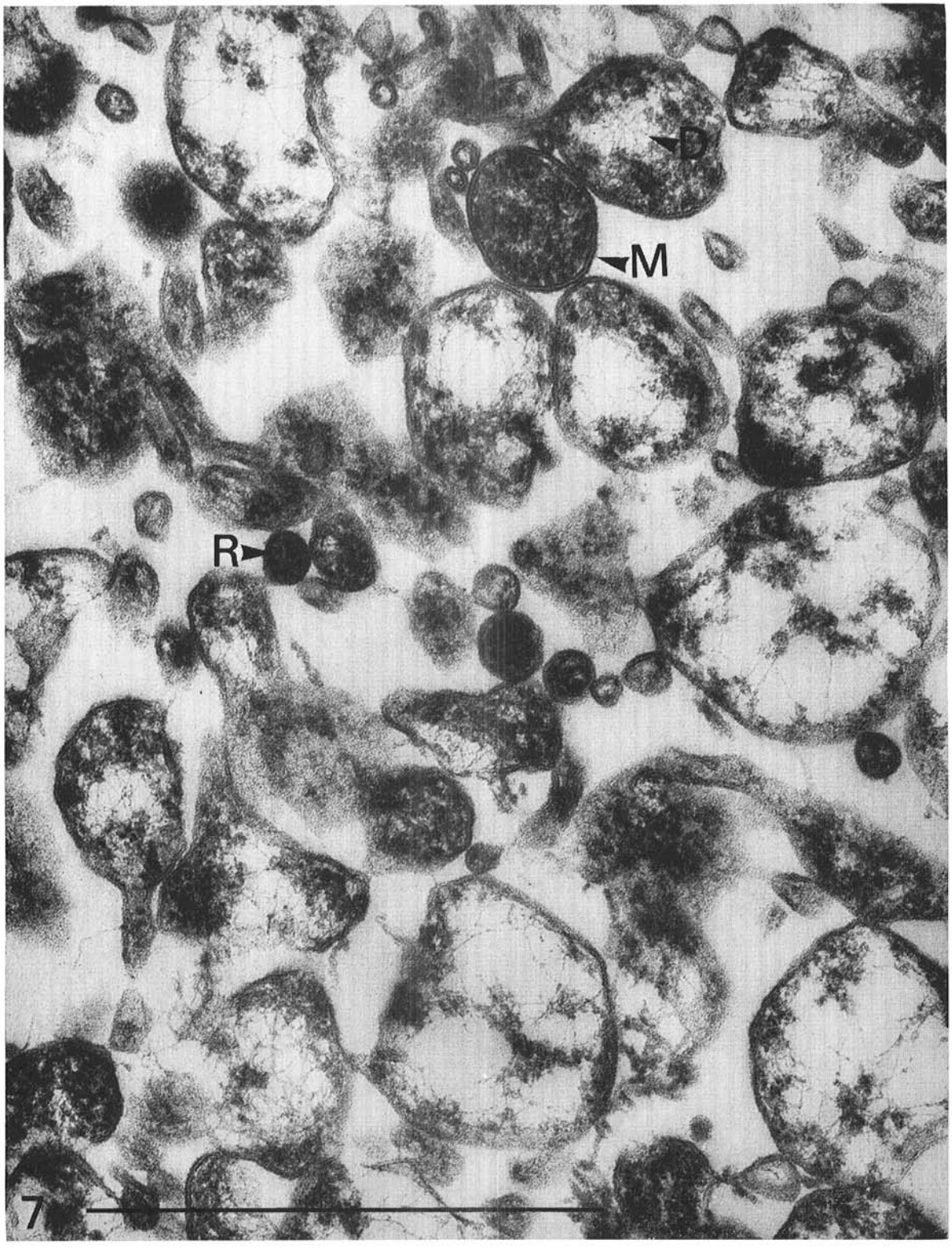


**Fig. 7.** High magnification micrograph showing mycoplasmalike bodies bound by trilaminar membrane (M). Small electron-dense bodies contain ribosomes (R) and larger bodies contain strands resembling DNA (D). Bar equals 1 μ.

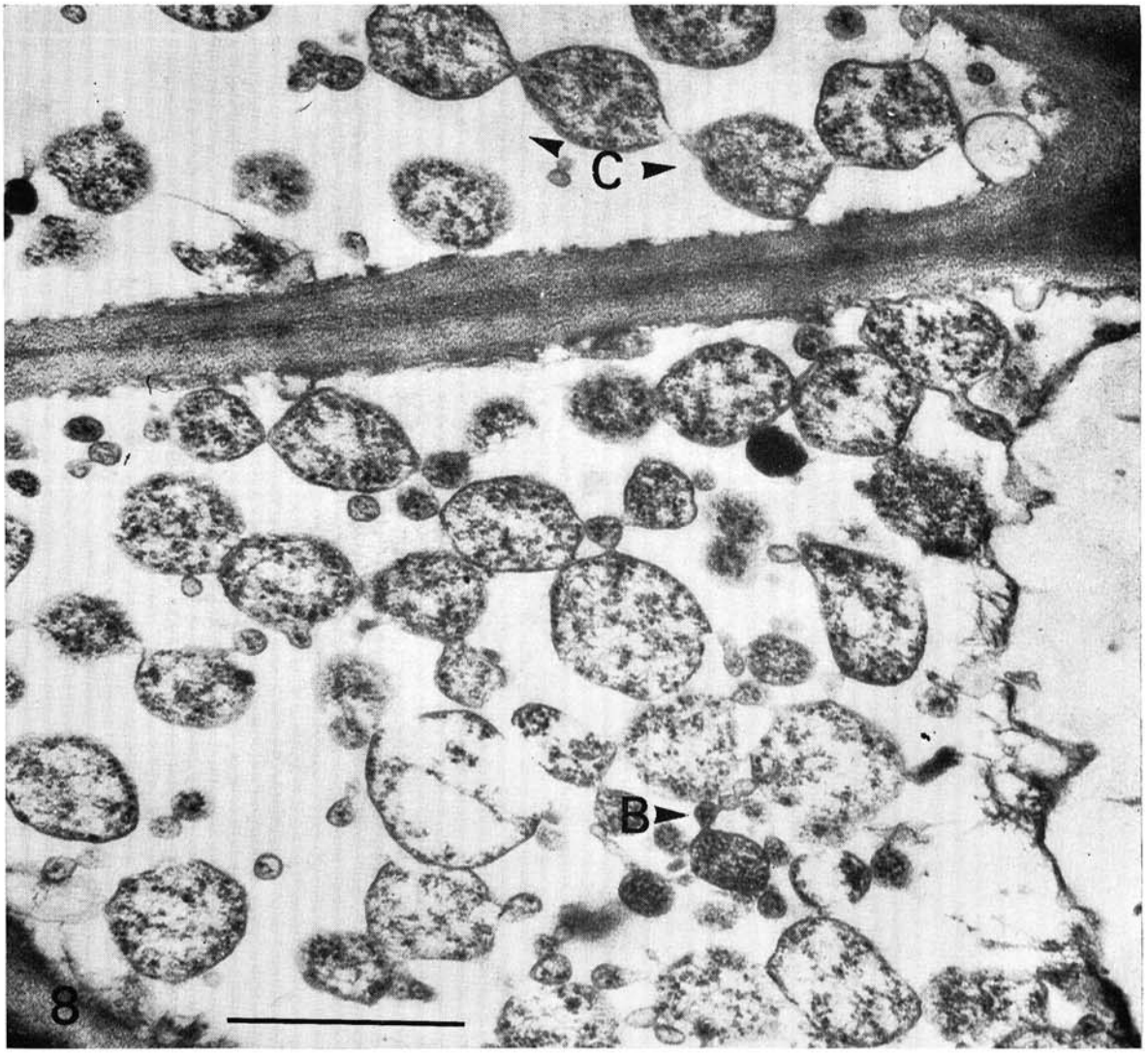


**Fig. 8.** Mycoplasmalike bodies showing chainlike configuration (C) and budding (B) in sieve elements of aster plant infected with aster yellows disease. Bar equals 1 μ.

plasmalike bodies in insect vectors carrying the aster yellows agent. On the other hand, no sure evidence of this form was seen in two plant species infected with the same agent, nor have they been described in any host tissue other than salivary glands of leafhoppers carrying aster yellows. In plants with aster yellows, this study revealed only the various forms described by Freundt (5). The structure shown in Fig. 4, for example, possibly represents bodies joined by filaments. Chains, the next stage of growth described by Freundt (5), were abundant (Fig. 1, 2).

Admittedly, attempts to piece together the reproductive cycle of an organism by sectioning infected host material is not an ideal approach. A tentative in vivo reproductive cycle of a mycoplasmalike organism in a plant host might, however, be proposed by drawing heavily upon existing information concerning growth of known mycoplasmas in vitro. Thus, for the mycoplasmalike organism in aster yellows-infected plants, spherical bodies may produce filaments that constrict to form chains of small bodies which then enlarge. The enlarged bodies may either form additional filaments, or reproduce by budding or by fission, and under adverse conditions they may collapse to form dense elongated forms.

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