

# OBSERVATIONS ON THE RELATIONSHIP BETWEEN *MYCOPLASMA*-LIKE BODIES AND HOST CELLS OF LEGUME LITTLE LEAF-DISEASED PLANTS

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## Summary

When sections of the abnormal green petals and ovary of flowers from cowpea [*Vigna sinensis* (L.) Savi ex Haask] affected by legume little leaf disease, were examined by electron microscopy, numerous *Mycoplasma*-like bodies were observed in certain sieve tube elements. Because of the location of some of the bodies in sieve plate pores, it is suggested that they pass between sieve tube elements of the phloem via the sieve pores. Presumably this movement is involved in the systemic infection of the plant.

Other observations indicated that at least at some stage of the infection, the bodies are present in intact cytoplasm, along with apparently normal organelles of the host cell. This observation contrasts with previously published electron micrographs of *Mycoplasma*-like bodies in plants affected by yellows diseases. These micrographs have shown the bodies only in cells apparently undergoing cytoplasmic degeneration, or in cells completely devoid of cytoplasm. If the bodies are the aetiological agents of legume little leaf, the occurrence of them in intact cytoplasm suggests a more highly specialized host-parasite relationship than previous results have indicated.

## I. INTRODUCTION

Legume "little leaf" was described by Hutton and Grylls (1956) as a disease affecting many tropical and subtropical legume species in Australia. On the basis of disease symptoms and modes of transmission, it was considered to be caused by a virus. However, Bowyer *et al.* (1969) were unable to detect virus particles in diseased plant tissue, but they observed numerous *Mycoplasma*-like bodies in sections of phloem tissue of plants affected by little leaf and other yellows-type diseases.

*Mycoplasma*-like structures were first described in thin sections of yellows-diseased plant tissue by Doi *et al.* (1967). There have since been several reports of similar structures in plants affected by various yellows diseases and in the cells of the leafhopper vectors of some of these diseases (Giannotti, Devauchelle, and Vago 1968; Giannotti, Marchou, Vago, and Duthoit 1968; Gourret and Maillet 1968; Granados, Maramorosch, and Shikata 1968; Lin and Lee 1968; Maillet, Gourret, and Hamon 1968; Maramorosch, Shikata, and Granados 1968; Hirumi and Maramorosch 1969). In the case of diseased plant tissue, the bodies have always been reported to occur in the sieve tubes of the phloem. In addition, there are three reports of the bodies in the phloem companion cells (Giannotti, Devauchelle, and Vago 1968; Lin and Lee 1968; Ploaie and Maramorosch 1969), and one report of

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them in the phloem parenchyma (Doi *et al.* 1967). The latter authors suggested that the bodies pass between the sieve tubes of the phloem via the pores of the sieve plates, since they observed the bodies in adjacent cells on each side of a sieve pore.

The precise nature of the *Mycoplasma*-like bodies described in diseased plants and insect vectors has not yet been established, nor has it been proven that they are the aetiological agents of the diseases involved. For these reasons, in this work we shall continue to refer to them as "*Mycoplasma*-like bodies", although the available evidence (discussed later) indicates that they are indeed *Mycoplasma*, and that they cause legume "little leaf" and other yellows diseases.

In this paper, cytological evidence supporting the possibility of movement of *Mycoplasma*-like bodies through sieve plate pores is presented, together with some other details of the relationship between the bodies and the infected phloem cells. This relationship included *Mycoplasma*-like bodies in (1) sieve tube elements devoid of cytoplasm, (2) in cells containing only cytoplasmic remnants, and (3) in cells with intact cytoplasm and apparently normal organelles.

## II. MATERIALS AND METHODS

Naturally infected shoots of a species of *Vigna* with typical symptoms of "little leaf" disease were obtained from Townsville, Qld. The plants presumably were infected via the known leafhopper vector, *Orosius argentatus* Evans (Hutton and Grylls 1956). Scions of the diseased material were cleft-grafted onto young cowpea plants [*Vigna sinensis* (L.) Savi ex Haask cv. Blackeye], in which symptoms appeared after 26–37 days of glasshouse culture. The characteristic green flower condition developed 43–48 days after grafting. The green petals, when fully expanded, and the hypotrophied ovary of one flower, were used for electron microscopy.

Strips of vein tissue were excised from the green petals, cut into small fragments, and fixed in 0.1M cacodylate-buffered 4.1% (0.41M) glutaraldehyde (pH 7.4) at 4°C for 20 hr. They were washed overnight in buffered 0.2M sucrose, then post-fixed in 1% (0.04M) osmium tetroxide for 20 hr. The tissue pieces were then dehydrated through a graded ethanol series, and propylene oxide, before embedding in Epon. The hypotrophied ovary was cut into small pieces for similar processing. Strips of the vein tissue of normal petals and ovary from healthy cowpea were prepared as control material. Sections were cut on a Porter–Blum ultramicrotome, mounted on carbon-coated copper grids, and stained with 5% (0.12M) aqueous uranyl acetate and lead citrate (Reynolds 1963). They were examined in a Siemens Elmiskop IA electron microscope.

## III. RESULTS AND DISCUSSION

### (a) Presence of *Mycoplasma*-like Bodies in Sieve Pores

Almost all sections of the green petal tissue revealed numerous *Mycoplasma*-like bodies in sieve tube elements. Sieve plates were frequently observed in the infected phloem, but only rarely (approximately in one sieve plate out of 20) was a *Mycoplasma*-like body seen in a sieve pore.

Figure 1\* shows a sectioned sieve plate associated with sieve tube cells of phloem tissue, with *Mycoplasma*-like bodies on both sides of the sieve plate. The characteristics of these bodies have been described previously (Bowyer *et al.* 1969). In

\* The following abbreviations are used on Figures 1–7: *M*, *Mycoplasma*-like body; *SE*, sieve element; *S*, sieve plate; *SA*, sieve area; *SP*, sieve pore; *MI*, mitochondrion; *N*, nucleus; *NL*, nucleolus; *NM*, nuclear membrane; *PM*, plasmalemma; *R*, ribosomes; *G*, Golgi body; *ER*, endoplasmic reticulum; *CGS*, cytoplasmic ground substance; *W*, cell wall.

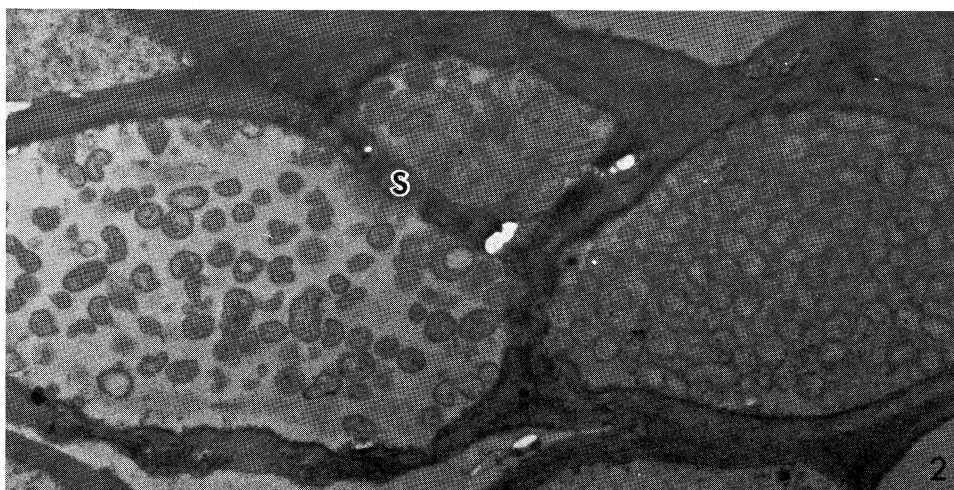
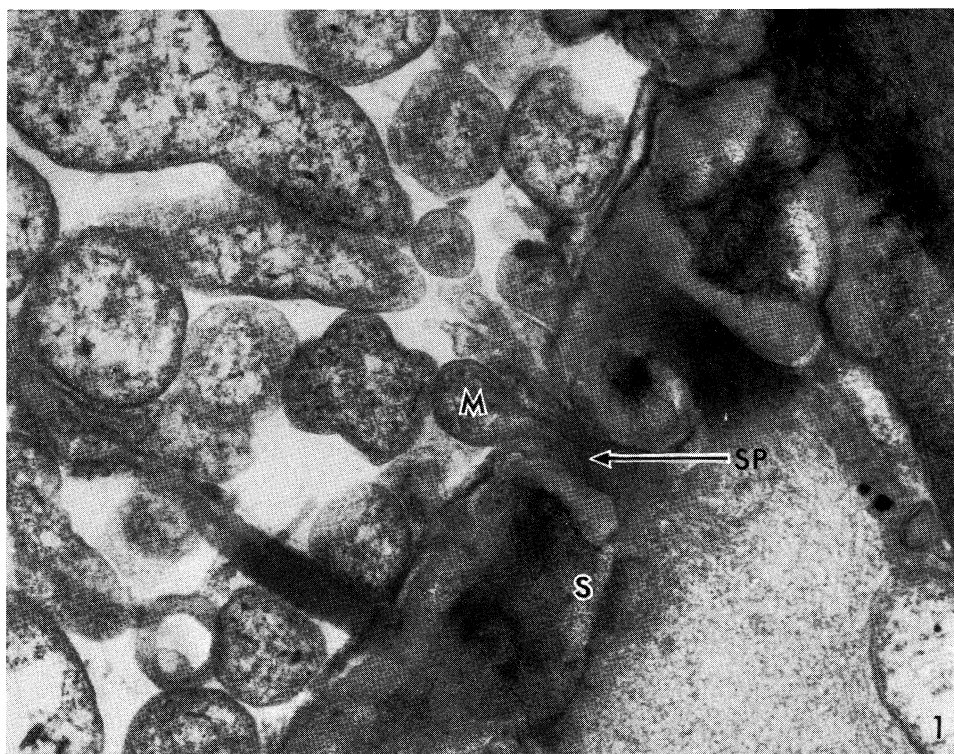


Fig. 1.—Apparent passage of a *Mycoplasma*-like body through a sieve pore in phloem tissue of a green petal of cowpea; pore completely sectioned, apparently constricting a body, which extends half way across the pore (arrow).  $\times 40,000$ .

Fig. 2.—Transverse section of a group of sieve elements in the phloem of the hypotrophied ovary from a green flower of cowpea. Size and location of the smallest cell indicate that it is a companion cell to the adjacent (left) sieve element, but the sieve plate distinguishes it as another sieve element. Note the numerous *Mycoplasma*-like bodies in each cell.  $\times 8,000$ .



Figure 1, a sieve pore has been completely sectioned, and a *Mycoplasma*-like body can be seen partially within, and apparently constricted by, the pore. Constriction of the body would presumably be necessary for its movement through the pore, since the latter has a diameter of approximately 130 nm, and that of the body at its broadest part is approximately 250 nm. It is conceivable that constriction readily occurs as the bodies enter a sieve pore, since they are bounded simply by a membrane, rather than a rigid outer wall. The characteristic pleomorphy and plasticity of *Mycoplasma* are attributed to this property, which apparently allows the organisms to pass filters of pore size smaller than the dimensions of the cells (Klieneberger-Nobel 1962).

All reports to date of *Mycoplasma*-like structures in plants indicate that they are restricted to phloem tissue. From observations based on Figure 1, it is envisaged that the bodies are transported along the sieve tubes in the sap, and that when they encounter a sieve area, some of the smaller bodies at least pass from one sieve tube element to the adjacent one via the sieve pores. If the bodies are indeed the aetiological agents of little leaf and other yellows diseases, it is suggested that they cause systemic infection of the plant in this way. The concept of such movement can be reconciled with evidence for the translocation of plant sap in the sieve tubes (Thaine 1961; Weatherley 1962).

Passage of the bodies through sieve pores would presumably be restricted to immature sieve elements, since during the maturation process deposits of callose are formed in the pores. However, progressive movement between immature elements would be sufficient to ensure eventual systemic infection. The concept of a progressive infection of the phloem in this way is supported by the fact that when little leaf is graft- or dodder-transmitted to *Nicotiana glutinosa* L., shoots immediately above and below the graft develop symptoms first, followed in succession by those located higher up the stem (Bowyer, unpublished observations).

The apparent passage of *Mycoplasma*-like bodies through sieve pores, as observed in this work, is paralleled by recent electron microscopic evidence for the movement of plant virus particles through sieve pores (Esau, Cronshaw, and Hoefert 1967). Giannotti, Devauchelle, and Vago (1968), Lin and Lee (1968), and Ploaie and Maramorosch (1969) have claimed that *Mycoplasma*-like bodies occur in the companion cells of yellows-diseased plants. If this is so, they presumably pass from sieve tube elements to the associated companion cells via the plasmodesmata, which are apparently involved in the intercellular translocation of certain plant viruses (Esau, Cronshaw, and Hoefert 1967; de Zoeten and Gaard 1969). Sieve tube elements are connected with the companion cells by plasmodesmata, but not by sieve pores (Frey-Wyssling and Müller 1957). The claims that *Mycoplasma*-like bodies occur in companion cells of the phloem in yellows-diseased plants are not supported by any evidence that the infected cells in question are companion cells. If size and relative

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Fig. 3.—Longitudinal section through a group of sieve elements of a green petal of cowpea. Note the very large numbers of *Mycoplasma*-like bodies in the three elements in the upper half of the micrograph—the lumen of the middle sieve element is occupied entirely by the bodies (*M*). Note the absence of cytoplasm in the infected sieve elements.  $\times 4,000$ .

position were the criteria used, in our experience this would be an unreliable basis. This is illustrated by Figure 2, in which the size and location of the smallest cell relative to the larger (left) cell would indicate that it is a companion cell, were it not for the associated sieve plate, which serves to characterize the cell as a sieve tube element. Had the sieve plate not been sectioned, the identity of the small cell would be uncertain. This illustrates the fact that the presence of *Mycoplasma*-like bodies in small cells adjacent to sieve tube elements cannot be accepted as evidence for the occurrence of the bodies in companion cells, particularly since it is known that companion cells are sparse among the abnormally numerous sieve elements of the phloem in aster yellows-diseased plants (Rasa and Esau 1961). The present study has provided no indication that plasmodesmata serve as "intercellular bridges" for the movement of *Mycoplasma*-like bodies between sieve tubes and companion cells.

### (b) *Mycoplasma*-like Bodies in Sieve Tube Elements

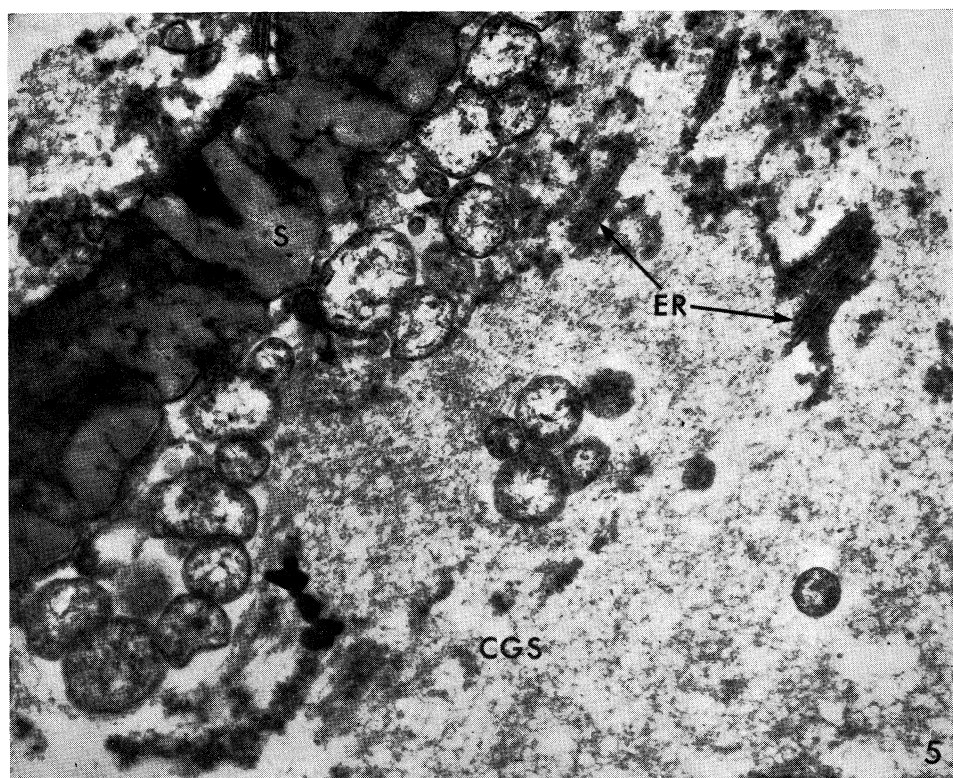
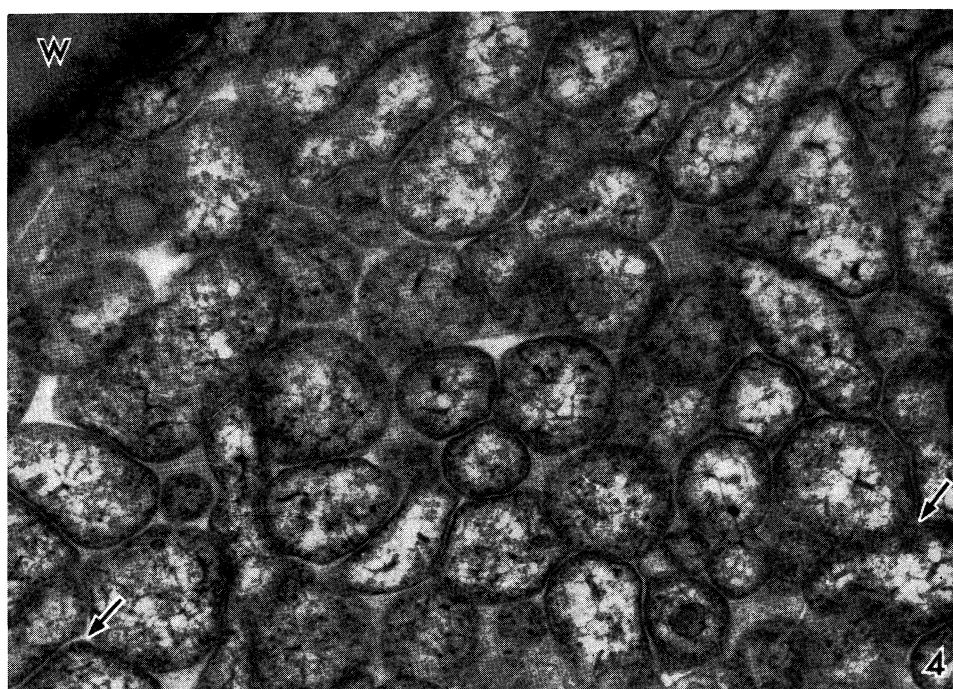
Figure 3 is a longitudinal section of a group of sieve tube elements containing numerous *Mycoplasma*-like bodies. It illustrates two interesting features:

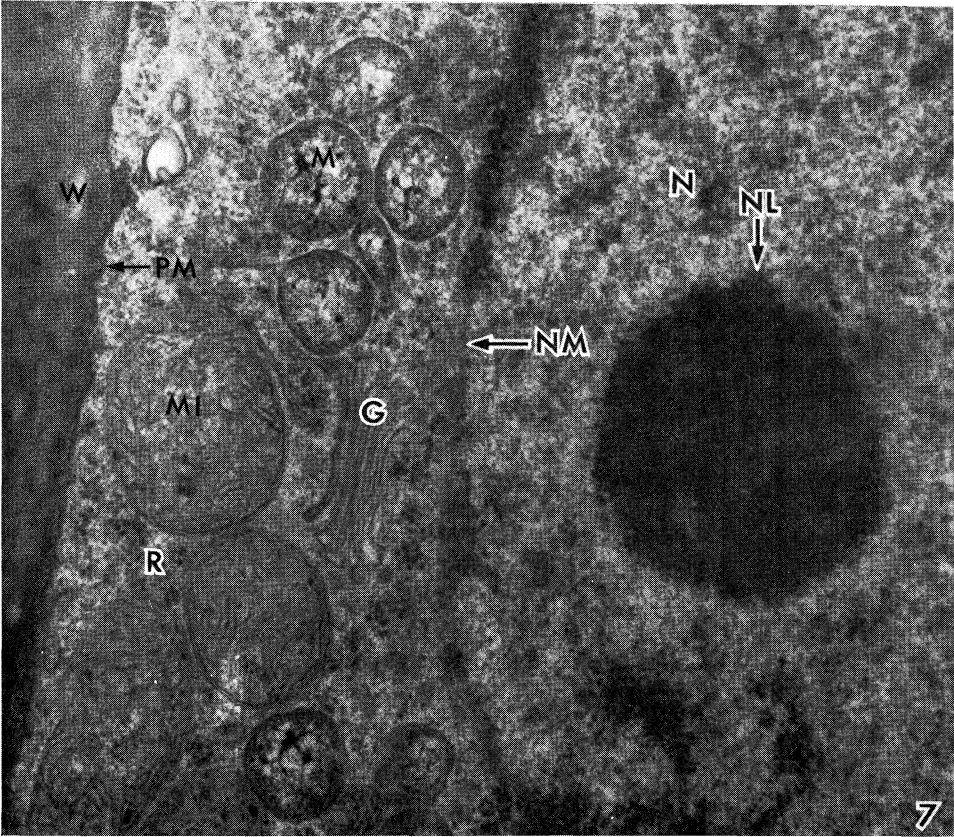
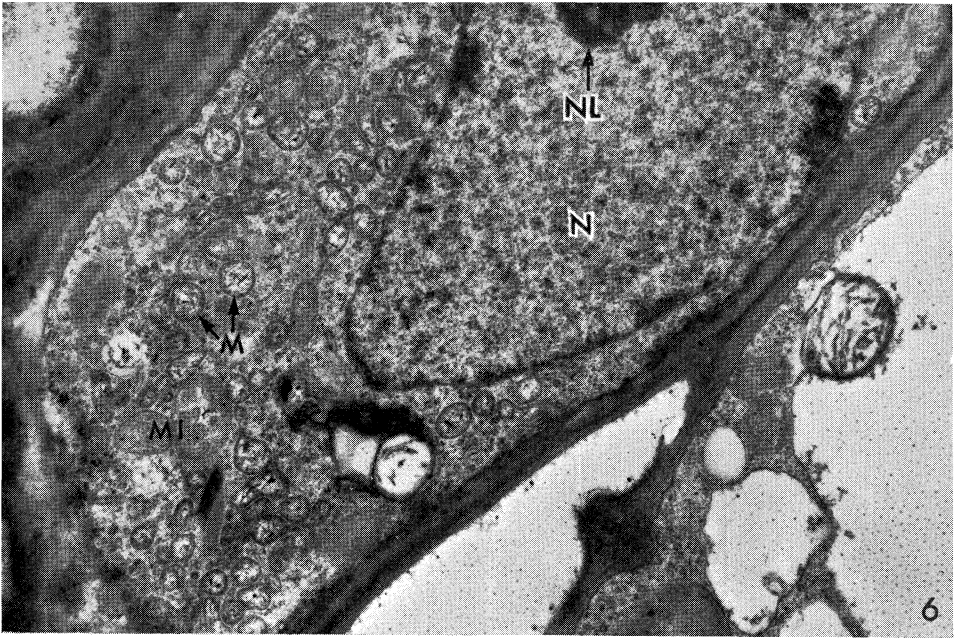
- (1) The extremely large numbers present in some cells, to the extent that the entire lumen of one cell is occupied by the bodies. Perhaps the phloem necrosis characteristic of yellows diseases (Rasa and Esau 1961) is associated with the apparent "plugging" of some of the sieve elements. This phenomenon might account for the presence of small, isolated areas of necrotic phloem in yellows-diseased plants.
- (2) The absence of cytoplasm in the infected cells, resulting in the appearance of hollow tubes containing only *Mycoplasma*-like bodies. This is the condition which has been most frequently observed, although occasionally the bodies have been found within sieve tube elements containing remnants of cytoplasm, including mitochondria, endoplasmic reticulum, and plastids (see Fig. 5). Since it is known that the nuclei of healthy sieve elements disintegrate during ontogeny (Esau 1960), it is not possible to determine if the absence of nuclei and cytoplasm in the infected sieve elements in Figure 3 is due to normal ontogenetic development, or if it is a consequence of the infection. The possibility that the nuclei of the infected cells were not included in the section is unlikely, since in similarly infected cells nuclei were never observed, but they were frequently seen in adjacent non-infected cells in the same section. It is also impossible to determine if infection occurred before or after any normal degenerative processes.

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Fig. 4.—Part of one of the sieve elements in Figure 3 at higher magnification. Note the orderly arrangement of the *Mycoplasma*-like bodies—small bodies occupy spaces among the larger ones, and where they "fit in" among adjacent bodies, the outlines of some are quite angular (arrows). The bodies range in diameter from approximately 70 to 900 nm.  $\times 40,000$ .

Fig. 5.—*Mycoplasma*-like bodies in a sieve element of the hypotrophied ovary of a green flower of cowpea. Note the presence of only a few bodies, scattered among remnants of the host cell cytoplasm, in contrast to Figure 3 where extremely large numbers are present in cells devoid of cytoplasm.  $\times 18,000$ .





However, the occasional presence of *Mycoplasma*-like bodies among remnants of host cytoplasm (Fig. 5), and the characteristic phloem necrosis associated with yellows diseases possibly indicate that premature cytoplasmic degeneration of phloem cells is a result of the infection. Figure 4 shows the arrangement of some of the bodies in one of the sieve elements in Figure 3, at higher magnification.

(c) *Mycoplasma-like Bodies Associated with Intact Protoplasts*

The great majority of sections of the green petals revealed *Mycoplasma*-like bodies in sieve tube elements devoid of protoplasts, as exemplified by Figures 1-3. By contrast, a few sections occasionally included cells in which the protoplasts were still intact, with *Mycoplasma*-like bodies occurring in the cytoplasm. Figures 6 and 7 show *Mycoplasma*-like bodies in the cytoplasm of phloem cells in which the normal organelles are present, and in which there is no evidence of protoplasmic degeneration. In Figure 6, numerous bodies are present in the cytoplasm, together with several mitochondria and the host nucleus. In Figure 7, a Golgi body and ribosomes are additionally present in the cytoplasm.

In view of the absence of sieve plates in both sections, it is difficult to determine the type of phloem cell involved, but the apparent secondary wall thickening of the infected cell in Figure 6 suggests that it is a sieve tube element. The presence of the host nuclei may be explained on the basis that the cells are young sieve elements in which nuclear disintegration has not occurred. It is possible that these cells are in an early stage of infection and that the protoplasts would degenerate during the course of the infection, resulting in the phenomenon observed in Figure 3. The relatively small number of *Mycoplasma*-like bodies in the cells in Figures 6 and 7 compared with the enormous numbers in Figure 3 might indicate such a development, that is, multiplication of the bodies associated with the degeneration of the protoplast of the host cell.

In any case, and irrespective of the nature of the cell type, Figures 6 and 7 firmly establish that *Mycoplasma*-like bodies occur in the intact cytoplasm of phloem cells from plants affected by a yellows disease. Gourret and Maillet (1968) regarded *Mycoplasma*-like bodies in *Vinca rosea*, infected by the agent of clover phyllody, as being extracellular, "in a biological fluid—the elaborated sap". On the basis of this concept it was claimed that they may be distinguished from the generally intracellular Rickettsiae and Chlamydiaceae. However, although electron micrographs such as Figure 3 might support the concept of the bodies being extracellular, Figures 6 and 7 clearly establish that they are intracellular.

It may well be significant that the *Mycoplasma*-like bodies discovered in the tissues of certain leafhopper vectors of some yellows diseases also occur intracellularly among the host cytoplasm (Granados, Maramorosch, and Shikata 1968; Maillet, Gourret, and Hamon 1968; Hirumi and Maramorosch 1969; Bowyer, unpublished data).

The occurrence of *Mycoplasma*-like bodies in the intact cytoplasm of both diseased plant and insect vector cells, might indicate a more highly specialized relationship between these bodies and the host cells, than the earlier cytological evidence suggested. Thus the isolation of the agents *in vitro* might prove a very difficult task. Attempts thus far indicate that they will not grow on standard media used for animal mycoplasmata (Hirumi and Maramorosch 1969; Bowyer, unpublished data). Nevertheless, the response of diseased plants to tetracycline antibiotics (Ishii *et al.* 1967; Davis, Whitcomb, and Steere 1968; Bowyer, unpublished data), together with the occurrence of the bodies in diseased plants and leafhopper vectors, but not in healthy plants or non-infective leafhoppers, strongly suggest that the aetiological agents of legume little leaf and other yellows diseases are indeed mycoplasmata.

#### IV. ACKNOWLEDGMENTS

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#### Note Added in Proof

Since the submission of this paper, two papers have appeared reporting the isolation and growth *in vitro* of *Mycoplasma* from diseased plants. Hampton, Stevens, and Allen (1969)\* have cultured a *Mycoplasma* species from diseased pea. They were able to reproduce the original disease symptoms only by combined inoculation with the *Mycoplasma* and alfalfa mosaic virus, also purified from the diseased plants. The apparent restriction of the *Mycoplasma* cells to parenchymatous tissue, and the phenomenon of aphid transmission of the *Mycoplasma*–virus complex, contrast with the characteristics of the yellows diseases.

Lin and Lee (1969)† report the culture of *Mycoplasma* from sugar-cane affected by white leaf disease. A small proportion of healthy plants inoculated with the organism developed symptoms of the disease. The occurrence of the organisms in phloem tissue, and leafhopper transmission of the disease, characterize it as one of the yellows group.

\* HAMPTON, R. O., STEVENS, J. O., and ALLEN, T. C. (1969).—Mechanically transmissible *Mycoplasma* from naturally infected peas. *Pl. Dis. Reprtr* **53**, 499–503.

† LIN, S., and LEE, C. (1969).—White leaf disease of sugar cane. *Newsl. Sug. Cane Pl. Path.* No. 3. pp. 2–3.

