Evidence that Clover Club Leaf is Caused by a Rickettsia-like Organism

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ABSTRACT

Electron micrographs of thin sections of phloem tissues from periwinkle, *Vinca rosea alba*, and crimson clover, *Trifolium incarnatum*, showing symptoms of clover club leaf disease demonstrated the presence of organisms bounded by a double membrane, or membrane and wall system, each structure measuring approximately 8 nm in thickness. In cross sections of phloem, some bodies appeared roughly spherical, with a diameter of approximately 200 nm, whereas others were elongated, measuring about 2 μm in length and 200 nm in diameter. No such organisms were ever found in the tissues of healthy plants. A probability study showed that the chance that these organisms were associated with the diseased plants purely fortuitously was not greater than 1/924. This probability indicates that this organism is the causal agent of clover club leaf disease. The organism appears to be more closely related to the rickettsias than to the mycoplasmas or chlamydiae.

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Clover club leaf (CCL) is a plant disease of which the earliest symptoms in crimson clover, *Trifolium incarnatum* L., are the yellowing and clubbing of the young leaves, the opened leaves often showing uneven expansion and lateral chlorosis of the leaflets. These symptoms eventually are followed by the dwarfing and premature death of the entire plant. The disease is transmitted by the leafhopper vector, *Agalliopsis novella* (Say), and was first described by Black (3).

In periwinkle (*Vinca rosea alba* L.) the disease causes, among other symptoms, virescence of the petals—a symptom characteristic of diseases of the aster yellows type. Because of the similarities of some symptoms of CCL and those of the yellows-type plant diseases (2, 19, 20, 23, 29) it was suspected that the causal organisms might also be similar in nature. Since 1967, when several Japanese workers (11, 18) reported the presence of mycoplasma-like bodies in the phloem of plants infected with certain yellows diseases, and also the remission of symptoms of these diseases by the administration of tetracycline antibiotics, a mycoplasmal etiology for the yellows-type plant diseases has come to be widely accepted by plant pathologists. However, antibiotic treatments of crimson clover plants showing symptoms of CCL showed that penicillin was more efficient than achromycin in producing remission of symptoms (34) and the possibility that the pathogen of CCL might belong to the rickettsiae or chlamydiae was raised (33).

Therefore, an attempt was made to visualize the disease agent in thin sections of infected tissues, and, subsequent to the finding of rickettsia-like bodies in the phloem cells, a study was undertaken to determine the probability that these bodies represent the causal organism of CCL. A brief report of these results has been presented (33).

MATERIALS AND METHODS.—Material for examination was cut from the second or third internode from the shoot tips of periwinkle, *Vinca rosea alba* L., showing virescence and reduction of the flowers, and from the petioles of newly expanded clover leaves showing severe symptoms of CCL. The tissues were infiltrated under vacuum with cold 3% glutaraldehyde. They were then fixed in fresh glutaraldehyde for 2 hr, postfixed for 4 hr in 2% osmium tetroxide, followed by en bloc staining for 30 min with 2% aqueous uranyl acetate, and dehydrated using an ethanol-propylene oxide series before being embedded in a 3:7 Epon mixture. Sections were cut on a Reichert OMU 2 ultramicrotome using a diamond knife, and poststained with 2% Reynold's lead citrate and 3% aqueous uranyl acetate. The sections were then examined and photographed in a Hitachi HU-11B electron microscope. Control samples of healthy periwinkle and clover plants were treated and examined in the same way for purposes of comparison.

RESULTS.—*Visualization of rickettsia-like bodies.*—Identical organisms were found in the phloem cells of both the periwinkle plants and the crimson clover plants showing symptoms of CCL, but no organisms were found in sections of tissues taken from healthy plants.

In periwinkle, many rounded bodies were found, with an average diam of about 200 nm and which possessed a distinct double membrane, or membrane and wall system (Fig. 1, 2). The inner layer of the outer structure, which may be analogous to a wall, appeared thicker and darker than the outermost layer. The inner membrane, or plasma membrane, exhibited the typical unit membrane structure, but was not always as clearly resolved as was the outer wall. These two structures appeared to be of equal thickness, each measuring approximately 8 nm across, and were separated by a space of varying dimensions, of about 7 to 16 nm (Fig. 1, 2). The interior of the bodies was filled with a granular cytoplasm containing dark ribosome-like particles. A clear area within the cytoplasm containing faint strands, possibly representing DNA, could be seen in some of the bodies. Where many of the bodies were present, the phloem cells were often filled with a matrix of what appeared to be plant protein, from which the bodies themselves tended to be set off by a clearer area around them, giving a slight halo-like effect.

The area of the bodies seen in transverse sections of the vascular tissue is sometimes very uniform (Fig. 1) and this suggests that elongate organisms oriented with their long axis parallel to the longitudinal axis of the phloem cells have been cut in cross section. Elongated bodies, up to 2 μm in length, with the same appearance and with the same diameter as the rounded forms, were also seen. The total length of the organisms in longitudinal section may well be in excess of the 2 μm measured on some elongate bodies seen in transverse sections.

The bodies found in the phloem cells of crimson clover petioles presented essentially the same appearance as those found in periwinkle. Rounded, oval, and elongate outlines were seen in sections (Fig. 3, 4, 5), with the same double membrane, or membrane and wall system, granular cytoplasm containing ribosome-like bodies, and central strands of possible nuclear material (Fig. 3). The shape and indistinctness of detail at some tips of the elongated bodies indicates that these tips have been cut in oblique section or that the body is curved out of the plane of section (Fig. 3, 4, 5). Measurements of membrane thickness, diameter, and length of the elongated forms corresponded to those given for the bodies found in periwinkle.

Curved elongate forms with a beaded outline in section were seen in one instance (Fig. 5). These may represent either replication by the rounding up of masses of cytoplasmic material within the outer wall of the filament, or possibly are an artifact due to thin sectioning of an undulated filament.

Much more work was done on clover than on periwinkle, and two additional structures were observed in clover. One type of structure found in infected clover, but not in infected periwinkle, took the form of narrow filaments about 25 nm in diameter and 500 nm long with a single visible bounding unit membrane and no discernible internal

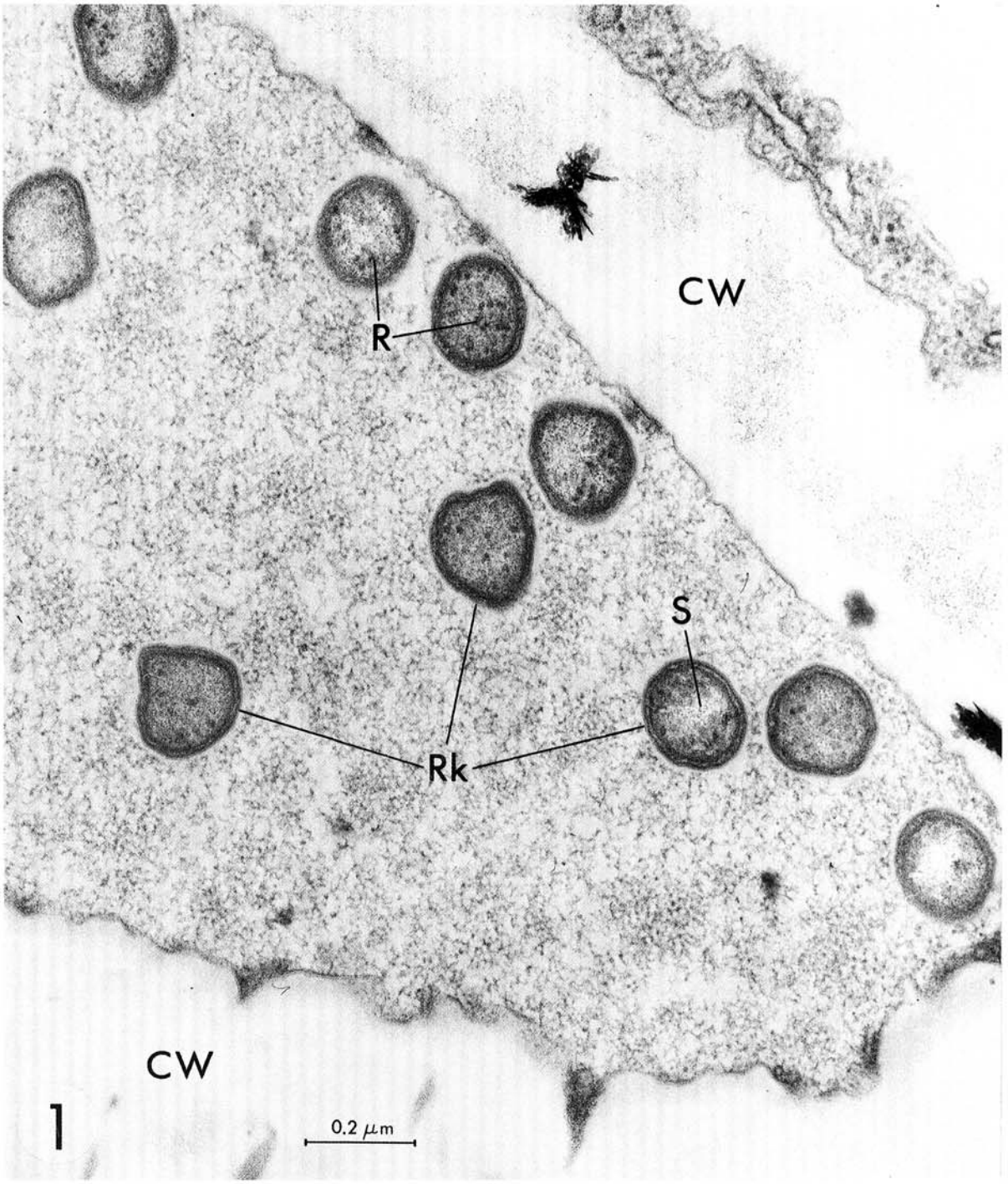

Fig. 1. Transverse section of a periwinkle shoot showing a phloem cell containing rickettsia-like (Rk) bodies limited by two trilaminar structures and showing ribosome-like particles (R). The central region is sometimes more electron translucent and may show fine strands (S), possibly of DNA. The rather uniform circular area of all the bodies suggests that they are transverse sections of elongated bodies oriented parallel to the shoot axis. CW = cell wall.

detail (Fig. 6). In one instance a cluster of very small rounded structures with the same diameter as a group of nearby filaments could be seen (Fig. 6) and these are thought to represent a cross-sectional view of such narrow filaments.

An outstanding difference between infected cells

of periwinkle and clover was the occasional presence in the latter of an additional rounded structure about 75 nm in diameter and apparently bounded by only a single unit membrane (Fig. 7, 8). No internal detail could be seen in these structures, but in some instances they gave the appearance of dividing (Fig. 8). In one or two cases it appeared as if a small structure was being produced by a blebbing of the

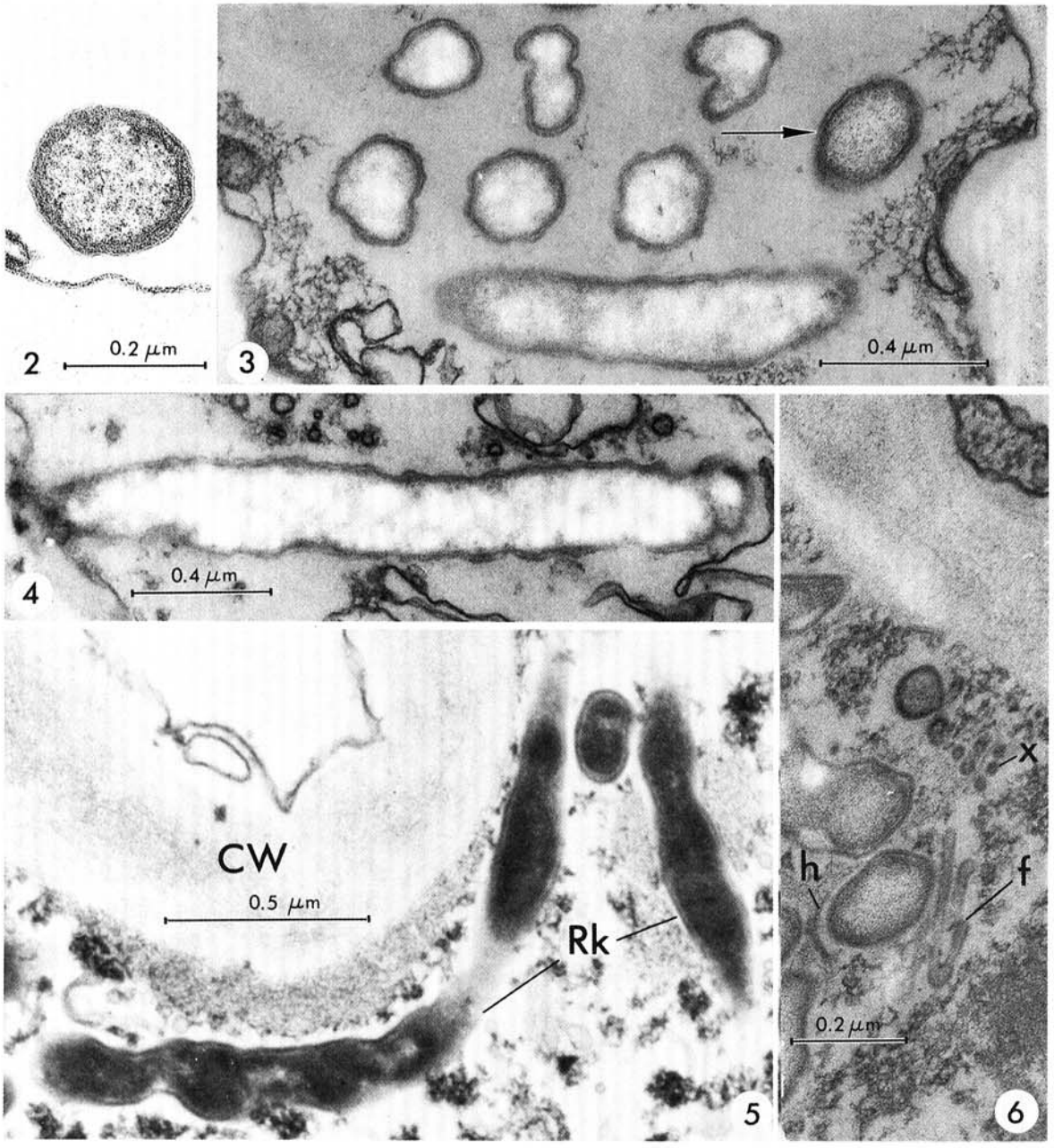

Fig. 2-6. 2) A rickettsia-like body in a periwinkle phloem cell. It shows two limiting trilaminar structures, the inner layer of the outer structure appearing heavier and darker than the outermost layer. 3) Rickettsia-like bodies in transverse section of phloem in a crimson clover petiole. They are limited by two structures. One body, indicated by the arrow, shows fine strands in the central region. At the ends of the elongate body the limiting structures are cut obliquely and are consequently not as clearly defined there as they are along part of the sides. 4) Rickettsia-like body about 2 μm long in a phloem cell of crimson clover. 5) Rickettsia-like bodies (Rk) in longitudinal section have a beaded appearance which may indicate rounding up of protoplasm within the outer wall or an undulated filament. From clover petiole. 6) Narrow filaments (f) were present with rickettsia-like bodies in some phloem cells. The small round structures (x) are thought to be transverse sections of the filaments. A definite halo (h) appears around one body. From clover.

outer membrane of a large rounded form, but this was by no means certain. Similar small structures were never observed alone in the cells of healthy crimson clover.

Association of clover club leaf symptoms with rickettsia-like bodies.—It was hypothesized that a rapid increase in the population of the pathogen occurred in infected parts shortly before the appearance of symptoms and that for some interval before the appearance of symptoms in new growth,

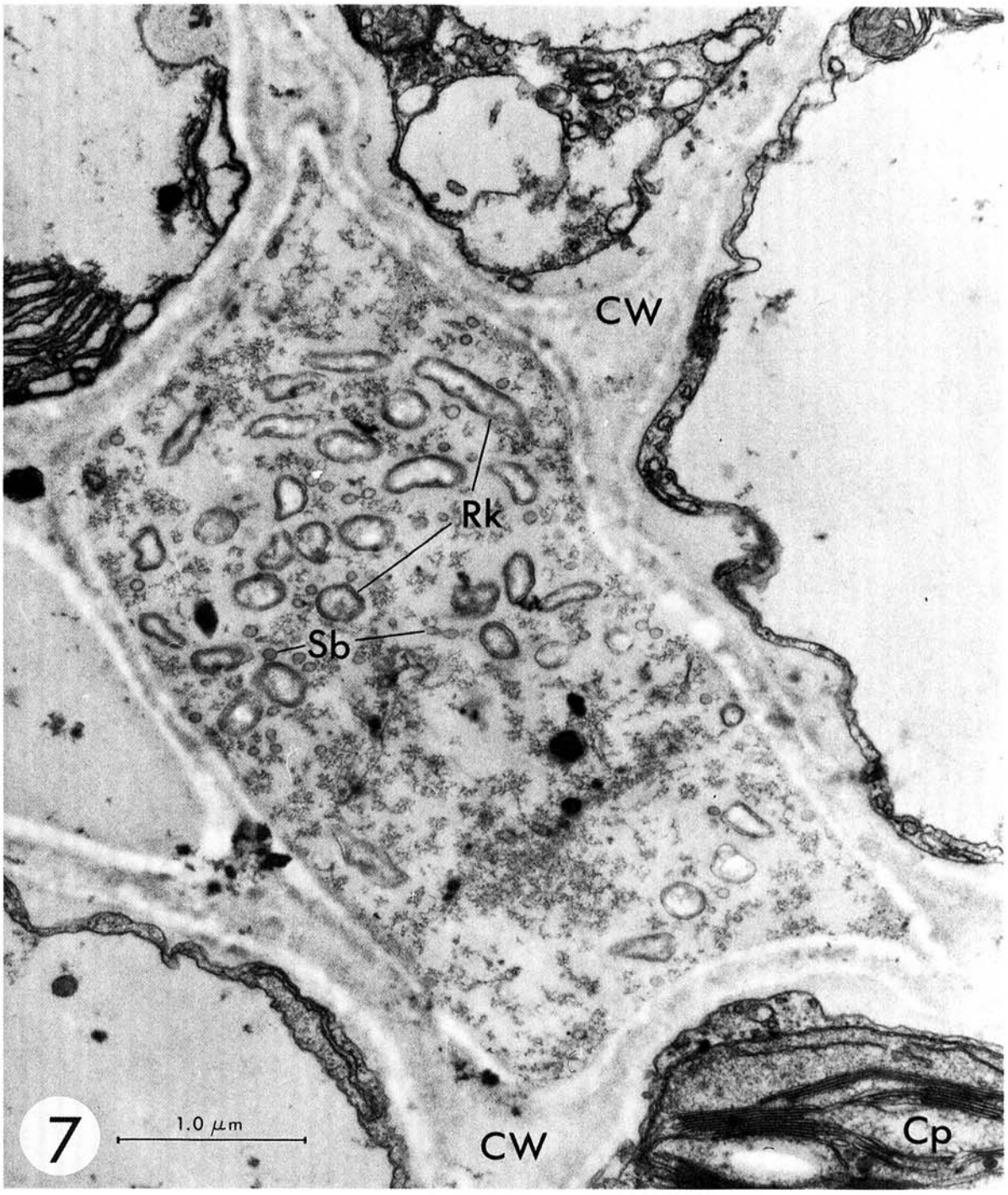

Fig. 7. A clover phloem cell showing many rickettsia-like bodies (Rk) and also many small structures (Sb). One of the neighboring cells shows part of a chloroplast (Cp).

where symptoms always first appeared, the pathogen would be absent, or present in such small numbers as to be undetectable by examination under the electron microscope. Accordingly, it was planned to compare parts that had recently developed symptoms with parts of control plants that had been treated in the same way but which failed to develop symptoms for at least two weeks after the parts were sampled.

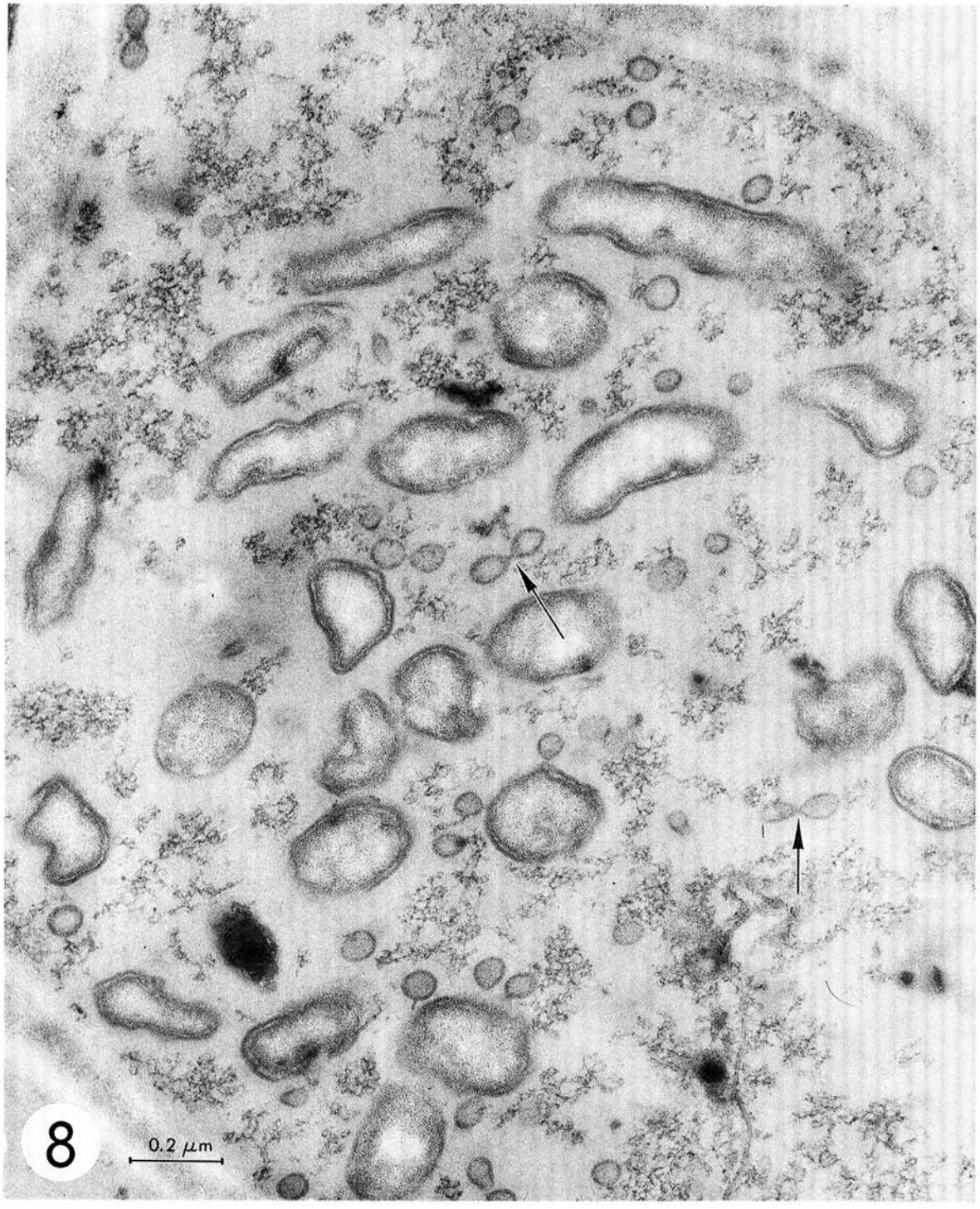

Fig. 8. Enlargement of part of Fig. 7 shows that the small structures have a single membrane. Some of the small structures indicated by arrows, appear to be dividing.

A group of healthy seedlings were exposed to inoculative insects for a period that would result in about half of them becoming diseased. An inoculative colony in which virtually all insects were carrying the agent had been identified in an effort to obtain agent-free insects by testing females from inoculative colonies individually on crimson clover plants. Each female was transferred once a week to a fresh clover seedling. Progeny from the females were saved and, if a sufficient number of progeny were obtained, five colonies of five nymphs from each female were tested on clover seedlings and transferred every 2 weeks to fresh seedlings. Such prolonged testing was necessary because transmission of the agent to clover is an infrequent event and test plants must be observed in the greenhouse for at least 10 weeks. Of 50 females tested adequately in this manner, none was found free of the agent (32).

Five insects from such inoculative colonies were placed on each of 20 crimson clover seedlings every week and allowed to feed on them for one week. When plants so exposed were kept in a greenhouse at 24 to 30 C, approximately 50% of the plants became diseased in 5 to 10 weeks. At the time the plants were chosen for the probability experiment the rate of appearance of diseased plants in certain inoculated batches was decreasing to such an extent that most of the exposed but still symptomless plants could be expected not to develop symptoms of club leaf within the two weeks following the day (day X) on which samples were taken for killing and fixing. All diseased plants chosen had developed symptoms within a period of 11 days before day X. It was planned that if any symptomless plant sampled developed symptoms within two weeks after day X its sample would be discarded and replaced by a sample from another symptomless plant without the knowledge of the person making the examinations under the electron microscope. Only one sample had to be discarded for this reason.

Tissues from eight exposed diseased plants and eight exposed symptomless plants were fixed and embedded in a single procedure on day X. The schedule for fixation and embedding was identical to that described in Materials and Methods. The samples were taken from the petioles of young newly expanded leaves showing severe symptoms, in the case of the infected plants, and from the petioles of leaves in an equivalent position on the exposed but symptomless plants. The plants were then returned to the greenhouse for observation for at least two weeks to determine if any symptoms developed in the eight symptomless exposed plants.

Three population sampling patterns were planned, in which the ratios of diseased-exposed to symptomless-exposed plant samples were 7:5, 6:6, and 5:7. By another random choice procedure, one of these patterns, which turned out to be the 6:6 ratio, was selected. The samples were given new symbols so that their identity was known only to investigator, L. M. Black (LMB). Samples were supplied in random order to investigator, I. M. Windsor (IMW), who did not know the population pattern chosen and who later determined the presence or absence of the characteristic organisms in each sample.

The samples were thin-sectioned and post-stained as before, and the sections scanned at a magnification of approximately X 10,000 for the presence of bodies similar to those previously found in the phloem cells of infected plants. In every case where rickettsia-like bodies were found, they were observed in the first grid, and in every subsequent grid examined. For samples where no rickettsia-like bodies were reported, at least three grids densely covered with sections were carefully scanned before concluding that no bodies were present.

The results were transmitted to and recorded by LMB before examination of the next sample. IMW did not know whether any of the results corresponded to the presence or absence of symptoms until all 12 determinations had been made. Bodies were found only in the six plants with symptoms. The random order in which the samples were supplied to IMW was +−++−−+−−++− where + indicates symptoms and bodies, and − indicates neither.

An interesting feature of this study is that one of the symptomless exposed plants began to show symptoms of CCL 21 days after day X. By chance when the symptoms developed its tissues had already been examined under the electron microscope and no bodies had been seen although the plant was obviously undergoing an incubation period at the time of sampling. This result indicates that the hypothesis regarding the low concentration of the pathogen some days before appearance of symptoms was true for at least one 21 day period. Actually it might be true for a period considerably less than the 14 days hypothesized as a safe period for the purpose of the experiment.

None of the other five symptomless exposed plants developed CCL symptoms during the observation period, which was extended for two months from day X.

By subjecting the results to statistical analysis, the correlation between the presence of the rickettsia-like bodies in the phloem and the appearance of disease symptoms in the plant is shown to be highly significant. Because of the considerable amount of work involved in making a determination on each sample, particularly on the negative ones, and because each determination was either positive or negative it is worth calculating the exact probabilities involved rather than approximating them with the X^2 calculation. The probability of obtaining the results by chance are obtained from the following calculation:

$$P = \frac{6!6!6!6!}{0!0!6!6!12!} = \frac{1}{924}.$$

In other words, the chance that the correlation between the presence of the described bodies in the phloem cells of the fixed samples and the presence of CCL symptoms in the original crimson clover plants is purely fortuitous is not greater than 1/924.

This considerable probability indicates: (i) that clover club leaf is not caused by the feeding of *Agalliopsis novella*; (ii) that it is not caused by *A. novella* carrying the pathogen (each of the symptomless plants had been exposed for 1 week to five inoculative *A. novella*); and (iii) that the disease is caused only when the rickettsia-like organism is transferred by the vector to the plant.

DISCUSSION.—It is evident from our micrographs that the organisms found in periwinkle and crimson clover plants infected with CCL appear very different from the mycoplasmas shown in micrographs of phloem tissues from plants infected with other diseases of the yellows type (7, 11, 15, 25, 27, 31). The most striking difference is the presence of a double membrane, or membrane and wall system, in the organism associated with CCL. By definition, the mycoplasmas as a group are bounded only by a single unit membrane (16), so that on this point alone the organisms associated with CCL must be excluded from consideration as mycoplasma-like bodies.

Supporting evidence indicating that the CCL-associated organisms are not mycoplasmas is provided by the results of our antibiotic treatment experiments (32, 34). Penicillin at a concentration of 312 units/ml produced clear remission of symptoms in CCL-infected plants, although, except for *Mycoplasma neurolyticum*, concentrations of >20,000 units/ml are required to produce inhibition of the animal mycoplasmas (30). However, if the outer membrane structure of the CCL-associated organism is really analogous to a wall containing muramic acid, the efficacity of the penicillin treatments would be expected.

In order to make a closer identification of the organisms found in the phloem cells of CCL-infected plants, the micrographs obtained were compared with some recently published for known rickettsiae and chlamydiae. It became apparent that the CCL organism did not closely resemble the chlamydiae, which, although intracellular, typically undergo their developmental cycle within cytoplasmic vacuoles and exhibit a multiplicity of forms (12) not encountered in the CCL study. However, when the CCL micrographs were compared with those of known rickettsias, many similarities were at once recognized.

Spherical, elliptical, and elongated forms of *Rickettsia canada* in the same size range as that reported for the CCL bodies have been seen, the elliptical forms also shading off at the ends as if cut obliquely (8). The clear areas noted around some of the CCL bodies (Fig. 6) were somewhat similar to the halos around many of the *R. canada*. Elongate forms in Fig. 4 & 5 seem very similar to an elongate form of *R. prowazeki* (1). The large CCL organisms appeared similar to the bacterial forms of *Rickettsiella melalonthae* described by Devauchelle et al. (10), and the small single-membrane bound bodies associated with the larger CCL organisms in some micrographs (Fig. 8) may perhaps be related to the small bodies which they described as being formed by evaginations of the wall at some developmental stages. Small filamentous structures, such as those described in Fig. 6, were not seen in any other micrographs in the literature examined.

In general, it appears that the ultrastructure of the bodies found in CCL-infected plants resembles that of the rickettsiae as a group rather than that of the chlamydiae.

Rickettsial bodies have been described by Maillet (22) in the salivary glands of *Euscelis lineolatus*, the vector of clover phyllody, a disease of the yellows type. Although he did not find any rickettsias in diseased plants, he drew attention to the possibility of rickettsias parasitic in arthropods being passed to plants upon which they feed. In 1970, Gianotti et al. (13, 14) reported the presence of a rickettsia-like organism with two limiting membranes in *Cuscuta* sp. This appears to be the first report of such an organism in plants.

Laflèche & Bové (21) and Saglio et al. (28) reported that in "greening" disease of citrus the associated organism in the phloem has a more complex limiting membrane structure, which measures about 200 nm across, than mycoplasmas have. Also, the organism associated with "greening" was more filamentous than many mycoplasmas. They suggested that the organism perhaps represents a new class of plant pathogen near mycoplasmas but different from them. In all of these characters the "greening" organism and the rickettsia-like organism of clover club leaf are similar.

Recently, Hopkins & Mollenhauer (17) reported a rickettsia-like organism to be associated with Pierce's disease of grapes. This organism is about 0.4 to 0.5 μm wide by 1.0 to 3.0 μm long, has a well defined cell wall, and inhabits the tracheal elements of the plant. It seems to differ from the CCL organism in several respects.

Papers are also in press (24, 26) reporting the occurrence of a rickettsia-like organism in sugarcane infected with ratoon stunting.

There is evidence that the thallus of the CCL organism sometimes has a somewhat undulated form (Fig. 5). Davis et al. (9) found that the corn stunt organism produced helical filaments and this led them to search for a wall or other supportive structure in addition to a limiting unit membrane. They could demonstrate only a single triple-layered membrane about 100 angstroms thick.

In addition to the demonstrated morphological similarity between the rickettsias and the CCL-associated organism, there are other features common to both the rickettsias and the CCL agent. The CCL agent is known to be arthropod-transmitted, and to multiply in the vector, giving a lifelong symptomless infection with high efficiency of transovarial passage to the next generation (4, 5, 6, 32). Incidentally, CCL appears to be the only plant disease of the yellows type for which transovarial passage of the agent has been definitely proven. In this it resembles known rickettsias parasitic in arthropods in which they are nonpathogenic and in which they are passed transovarially from generation to generation. They also resemble known rickettsias in producing disease in the host upon which the

arthropod vector feeds. The rickettsias are small, highly specialized, gram-negative bacteria which are parasitic on arthropods. Most of them are nonpathogenic in their arthropod host, but some are transmitted by insect bite to man or other animals in which they are pathogenic. For instance *R. rickettsii*, the agent of Rocky Mountain spotted fever, is transmitted to man in the saliva of infected ticks which are themselves apparently unaffected by its presence and pass it to the progeny through the egg.

So far, it has not been possible to prove by the satisfaction of Koch's rules of proof that the organism described in CCL-infected plants is the causal agent of the disease. However, the results of the probability experiment, indicating that the possibility of a chance association of this organism with CCL-infected plants was not greater than 1/924, provide a very strong basis for considering it to be the causal agent.

It is concluded that the causal agent of clover club leaf disease is probably more closely related to the rickettsias than to any other group of organisms.

LITERATURE CITED

1. BIRD, R. G., N. KORDOVA, & J. REHACEK. 1967. Fine structure of Rickettsia prowazeki in the haemocytes of ticks, Hyalomma dromedarii. Acta Virol. 11:60-62.
2. BLACK, L. M. 1941. Further evidence for multiplication of the aster-yellows virus in the aster leafhopper. Phytopathology 31:120-135.
3. BLACK, L. M. 1944. Some viruses transmitted by agallian leafhoppers. Proc. Amer. Philos. Soc. 88:132-144.
4. BLACK, L. M. 1948. Transmission of clover club-leaf virus through the egg of its insect vector. Phytopathology 38:2 (Abstr.).
5. BLACK, L. M. 1949. Multiplication of clover club-leaf virus (Aureogenus clavifolium) in its insect vector. Phytopathology 39:2-3 (Abstr.).
6. BLACK, L. M. 1950. A plant virus that multiplies in its insect vector. Nature 166:852-853.
7. BOWYER, J. W., J. G. ATHERTON, D. S. TEAKLE, & G. A. AHERN. 1969. Mycoplasma-like bodies in plants affected by legume little leaf, tomato big bud, and lucerne witches' broom diseases. Aust. J. Biol. Sci. 22:271-274.
8. BRINTON, L. P., & W. BURGDORFER. 1971. Fine structure of Rickettsia canada in tissues of Dermacentor andersoni Stiles. J. Bact. 105:1149-1159.
9. DAVIS, R. E., J. F. WORLEY, R. F. WHITCOMB, T. ISHIJIMA, & R. L. STEERE. 1972. Helical filaments produced by a mycoplasma-like organism associated with corn stunt disease. Science 176:521-523.
10. DEVAUCHELLE, G., G. MENADIER, & C. VAGO. 1972. Étude ultrastructurale du cycle de multiplication de Rickettsiella melolonthae (Krieg), Philip, dans les hémocytes de son hote. J. Ultrastruct. Res. 38:134-148.
11. DOI, Y., M. TERANAKA, K. YORA, & H. ASUYAMA. 1967. Mycoplasma- or PLT group-like microorganisms found in the phloem elements of plants infected with mulberry dwarf, potato witches' broom, aster yellows, or Paulownia witches' broom. Ann. Phytopathol. Soc. Japan 33:259-266.
12. FRIIS, R. R. 1972. Interaction of L cells and Chlamydia psittaci: Entry of the parasite and host responses to its development. J. Bact. 110:706-721.
13. GIANOTTI, J., C. VAGO, G. MARCHOUX, G. DEVAUCHELLE, & J. L. DUTHOIT. 1970. Infection de plante par un type inhabituel de microorganisme intracellulaire. C. R. Acad. Sci. Ser. D. 271:2118-2119.
14. GIANOTTI, J., C. VAGO, J. SASSINE, & D. CZARNECKY. 1971. Culture et transmission de mycoplasmes phytopathogènes. C. R. Acad. Sci. Ser. D. 272:1776-1778.
15. GRANADOS, R. R., K. MARAMOROSCH, & E. SHIKATA. 1968. Mycoplasma: suspected etiologic agent of corn stunt. Proc. Nat. Acad. Sci. 60:841-844.
16. HAYFLICK, L. 1969. The Mycoplasmatales and the L-phase of bacteria. Chap. 2, p. 15-47. Appleton-Century-Crofts, New York.
17. HOPKINS, D. L., & H. H. MOLLENHAUER. 1973. Rickettsia-like bacterium associated with Pierce's disease of grapes. Science 179:298-300.
18. ISHIIE, T., Y. DOI, K. YORA, & H. ASUYAMA. 1967. Suppressive effects of antibiotics of tetracycline group on symptom development of mulberry dwarf disease. Ann. Phytopathol. Soc. Japan 33:267-275.
19. KUNKEL, L. O. 1924. Insect transmission of aster yellows. Phytopathology 14:54 (Abstr.).
20. KUNKEL, L. O. 1951. Yellows diseases of plants. Bull. Torrey Bot. Club 78:269-270.
21. LAFLÈCHE, D., & J. M. BOVÉ. 1970. Structures de type mycoplasme dans les feuilles d'orangers atteints de la maladie du "grenning". C. R. Acad. Sci. (Paris) Ser. D. 270:1915-1917.
22. MAILLET, P. L. 1970. Présence de particules de type rickettsien dans la salive d'un Homoptère vecteur de la phyllodie du trèfle, Euscelis lineolatus Brullé (Homoptera, Jassidae). Rev. Can. Biol. 29:391-393.
23. MARAMOROSCH, K. 1952. Direct evidence for the multiplication of aster-yellows virus in its insect vector. Phytopathology 42:59-64.
24. MARAMOROSCH, K., B. PLAVSIC-BANJAC, J. BIRD, & L. L. LIU. Electron microscopy of ratoon stunted sugar cane: microorganisms in xylem. Phytopathol. Z. (In press).
25. NASU, S., D. D. JENSEN, & J. RICHARDSON. 1970. Electron microscopy of mycoplasma-like bodies associated with insect and plant hosts of peach western-X disease. Virology 41:583-595.
26. PLAVSIC-BANJAC, B., & K. MARAMOROSCH. Phloem-infecting mycoplasmas and xylem infecting rickettsia-like organisms of two plant diseases. (English translation of Yugoslav title) Proc. 2nd Congress Yugoslav Microbiologists. Opatija. Sept. 1972.
27. PLOAIE, P., & K. MARAMOROSCH. 1969. Electron microscopic demonstration of particles resembling mycoplasma or psittacosis-lymphogranuloma trachoma group in plants infected with European yellows-type diseases. Phytopathology 59:536-544.
28. SAGLIO, P., D. LAFLÈCHE, C. BONISSOL, & J. M. BOVÉ. 1971. Isolement, culture et observation au microscope électronique des structures de type mycoplasme associées à la maladie du stubborn des agrumes et leur comparison avec les structures observées dans le cas de la maladie du Greening des agrumes. Physiol. Vég. 9 (4):569-582.
29. SMITH, R. E. 1902. Growing china asters. Hatch Exp. Sta., Mass. Agr. Coll. Bull. 79. 26 p.
30. WARD, J. R., S. MADOFF, & L. DIENES. 1958. In vitro sensitivity of some bacteria, their L-forms and pleuropneumonia-like organisms to antibiotics. Proc. Soc. Exp. Biol. Med. 97:132-135.
31. WILSON, C. L., C. E. SELISKAR, & C. R. KRAUSE. 1972. Mycoplasma-like bodies associated with elm

phloem necrosis. Phytopathology 62:140-143.
32. WINDSOR, I. G. 1972. Clover club leaf: a possible rickettsial disease of plants. Ph.D. Thesis. University of Illinois, Urbana. 135 p.
33. WINDSOR, I. M., & L. M. BLACK. 1972. Clover club-leaf: a possible rickettsial disease of plants. Phytopathology 62:1112 (Abstr.).
34. WINDSOR, I. M., & L. M. BLACK. 1973. Remission of symptoms of clover club leaf following treatment with penicillin. Phytopathology 63:44-46.