Three-Dimensional Structure and Morphology of Mycoplasmalike Bodies Associated With Albino Disease of Prunus avium

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ABSTRACT

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Mycoplasmalike bodies were found in association with albino disease of *Prunus avium* L. The bodies were present in mature sieve tube elements of diseased tissue but not in healthy controls. The bodies ranged from 0.15 to 1.0 μm in diameter and 0.5 to 1.8 μm in length. Morphologically the bodies were spherical or tubular. Some of the tubular bodies were branched, and some bodies were attached to stacked endoplasmic reticulum.

Additional key words: cherry.

Mycoplasmalike bodies (MLB) have been associated with more than 70 yellows diseases since Doi et al. (8) first proposed the hypothesis that 'yellows-type diseases' may be caused by a mycoplasma. Recently, Begtrup and Thomsen (2) reported MLB associated with seven genera of wildflowers showing yellows symptoms. These associations are mostly based upon electron microscopic observations of MLB in situ. However, information about the in situ development, three-dimensional structure, and intracellular relationships is lacking. Because of this, we initiated a developmental cytopathological study on albino-diseased *Prunus avium* L. This paper reports the results of that study.

MATERIALS AND METHODS

Three albino-diseased *P. avium* L. trees were identified in an orchard at Talent, Oregon, during the summer of 1971. Controls were a healthy virus-indexed tree maintained under screen at the Oregon State University Botany and Plant Pathology Research Facility, and several peach trees used in a comparative study of X-disease (9). Sampling of cherry trees started on 3 April 1972 and continued on a monthly basis until 7 September 1972, resuming on 6 April 1973, and ending on 1 August 1973. The distal 2-5 mm of leaf midvein and portions of peduncle were diced directly into 5% glutaraldehyde buffered to pH 7.0 with 0.125 M sodium phosphate buffer. Fixation was for 1-2 hr at room temperature followed by 10 hr at 4 C under vacuum. The tissue was buffer washed, postfixed in buffered osmium tetroxide 6-8 hr, dehydrated with acetone and propylene oxide, and embedded in Epon 812 (9). Serial thin-sections 60-90 nm thick were mounted on Formvar-coated, 149-μm parallel line, copper grids, and post-stained for 10 min in lead citrate pH 12.0, Reynolds (21). They were examined and serially photographed with a Phillips EM-300 electron microscope operating at 60 kV. Equal time was spent examining healthy and diseased tissue.

When pertinent sets of serial photographs were obtained, the photographic images from the negatives were enlarged onto 8 × 10 DuPont Cronar Ortho Litho Type S sheet film. This resulted in a positive image on a transparent film base. These transparencies were aligned and stacked using techniques outlined by Brown and Arnott (3), then stereo pair photographs were taken for subsequent study and recording of three-dimensional structure.

RESULTS

Mycoplasmalike bodies were observed in mature sieve tube elements of samples taken from diseased trees during June, July, and August (10). The bodies were present in mature sieve tube elements of both leaf and peduncle (Fig. 1, 2, 3). Vesicles in expanded endoplasmic reticulum cisternae and in cisternae formed by the outer nuclear membrane were observed in immature phloem parenchyma cells and sieve tube elements of albino-diseased *P. avium* (9). No virus particles were found, and comparable structures were not found in control trees at the same stage of development; i.e., in mature sieve tube elements from healthy trees. The structures ranged from 0.15 to 1.0 μm in diameter with a mean diameter of 0.41 μm ($P = 0.01$) indicating that the mean lies between 0.34 and 0.47 μm. Length ranged from 0.51 to 1.8 μm with a mean length of 0.96 μm ($P = 0.01$), indicating that the mean lies between 0.82 and 1.1 μm. The structures ranged from spherical (sph) to tubular (tu) in shape (Fig. 1-C and 3-H), were bound by a unit membrane, and usually contained a dense nucleoid area (Fig. 1).

The tubular and spherical forms are exemplified in the stereo pair photographs of Fig. 4. (Note: The reader must supply a viewer for the stereo pairs.) It should be noted that the diameter of the tubular forms ranges from 0.15 μm to 0.20 μm, which is at the small end of the range given

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for diameter. The spherical bodies have a diameter which coincides more closely to the mean. The tubular forms appear to be attached to stacked endoplasmic reticulum (ER). For example, in stereo pair 4-A, the tubular structure numbered 1 can be traced to the cell wall. In section 1-D (arrow), a connection (CN) to the ER is apparent. Also, in stereo pair 4-B (arrow) a connection is visible which can be seen in section 3-D. Other connections (arrows) are visible in sections 3-(E, G). In each case the connection appears in only one section and is therefore a very small point of attachment; i.e., less than the thickness of a single thin-section (60-90 nm). None of the spherical structures appears to be attached; however, some intermediate forms between tubular and spherical do occur and appear to branch. The structure marked by the arrow in Fig. 3-K is a composite structure of the components marked by arrows in serial sections 3-(C to J). Notice the Y-shaped branch in the structure in Fig. 3-K. This structure can also be seen in stereo pairs Fig. 4-(C, D). Figures 4-(C, D) represent reciprocal arrangements of the ten serial sections in Fig. 3-(A to J). The spherical structure in stereo pair 4-C (arrow) is located over tubular forms. In stereo pair 4-D the same structure can be seen under the tubular forms. There appears to be no interconnection between the spherical MLB and the adjacent tubular MLB. The same spherical MLB can be traced in section 3-(A to C) and no evidence of interconnection is visible.

DISCUSSION

The tubular MLB (Fig. 1 and 3) bear some resemblance to *Spiroplasma* reported recently (5, 6). Diameter measurements and structural components coincide most closely. However, serial sections show these MLB are shorter (i.e., 1.8 μm maximum length compared to 3-15 μm and have no helical structure. Davis et al. (6) saw whole spirals in 0.3 μm thick sections. Since thin sections range from 60-90 nm the sections in Fig. 1, 2, and 3 cover between 0.48 to 0.72 μm and 0.60 μm to 0.90 μm of a single cell, respectively. However, no spirals were observed in these sections and spirals were not observed

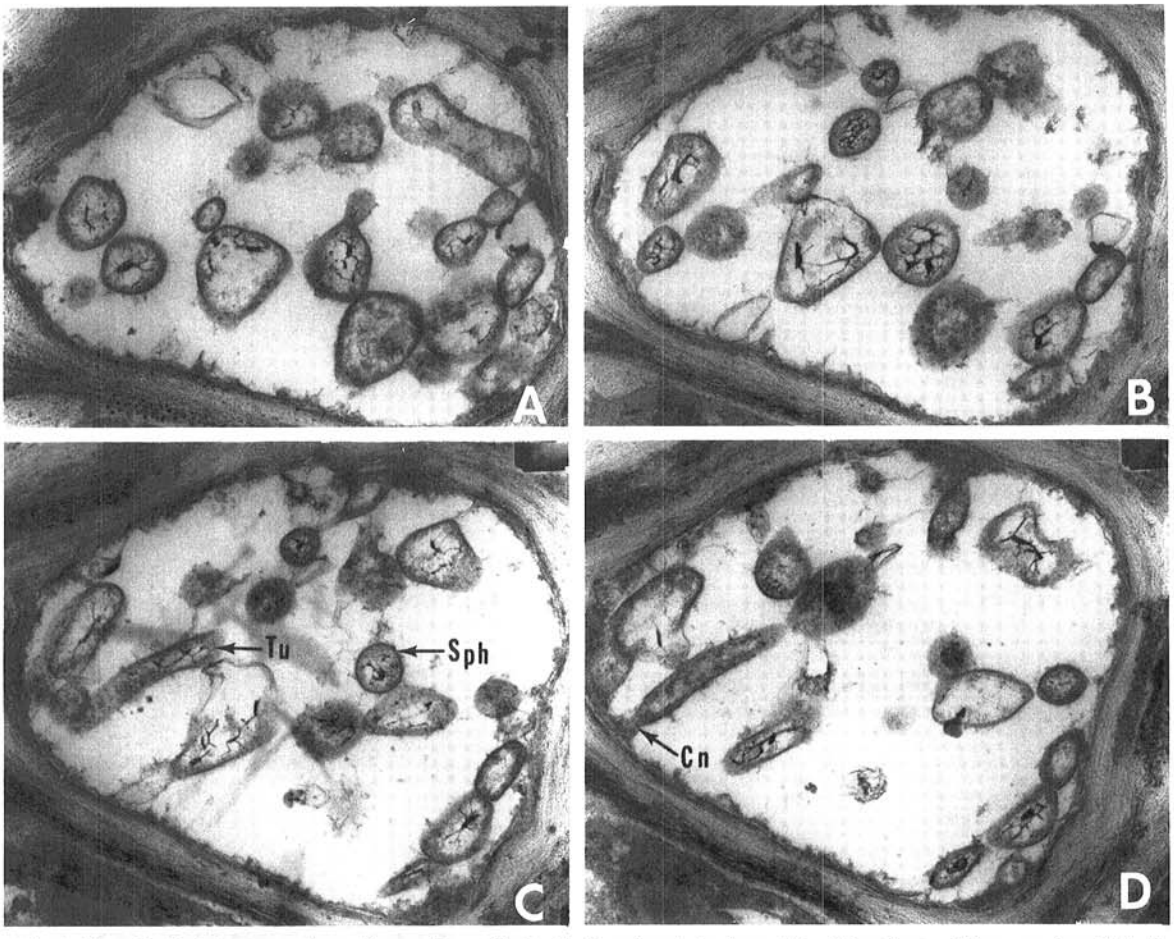

Fig. 1-(A to D). Serial cross sections of mycoplasmalike bodies in a sieve tube element in albino-diseased *Prunus avium*. Note the electron-dense fibrils contained within the mycoplasmalike bodies, tubular (tu) and spherical (sph) morphology, and the connection (cn) of the tubular MLB in section D (arrow). × 22,800 (1 mm = 44 nm). See composite stereo pairs of all sections from Fig. 1 and 2 in Fig. 4-A.

in expressed plant sap. Therefore, it is concluded that the tubular forms associated with albino disease of *P. avium* are not *Spiroplasma*.

Both tubular and spherical structures are quite different from rickettsialike structures reported by Goheen et al. (11) and Nyland et al. (19). The MLB in albino-diseased material is smaller and there is no evidence of a rippled cell wall. In addition, the structures always were found in mature sieve tube elements, whereas rickettsialike structures are found in vessels.

Based on symptomatology and transmission studies, albino disease of *P. avium* is very similar to X-disease of *P. persica* and *P. avium*. Yet there is considerable difference in morphology between the MLB observed in albino-diseased *P. avium* from Oregon and the MLB in X-diseased *P. persica* observed by MacBeath et al. (17) in California. They reported undulating tubules 5 μm in length packing sieve tube elements, whereas our data show tubules not exceeding 2 μm interspersed with spherical forms (Fig. 1-4). The variations may be due to the observance of different developmental stages or climatic variations, and/or strain differences. The structures in albino-diseased tissue also differ morphologically from the MLB reported by Nasu et al. (18) in the cells of *Colladonus montanus* and *Apium graveolens*, even though both were infected with X-disease agent. In this case, the structures in albino are larger. Nasu et al. (18) reported a size range of 200-400 nm, compared to 150-1,020 nm and up to 1,800 nm long in albino-diseased peach. The discrepancies may be due to different fixation methods, the fact that the X-disease agent was in two entirely different types of tissue, and that Nasu et al. (18) presented no evidence of serial sectioning so that overall form could not be determined.

Of the various structures reported to be associated with yellows diseases, the structures observed in albino-diseased tissue most closely resemble those reported by other researchers as mycoplasmalike bodies. However, they also closely resemble *Chlamydia* as described by Lepinay et al. (15, 16) and Cutlip (4), and the L-forms described by Hayflick (12).

One structural feature associated with albino-diseased tissue which has not been reported previously is the connection of the tubular forms at points along the cell

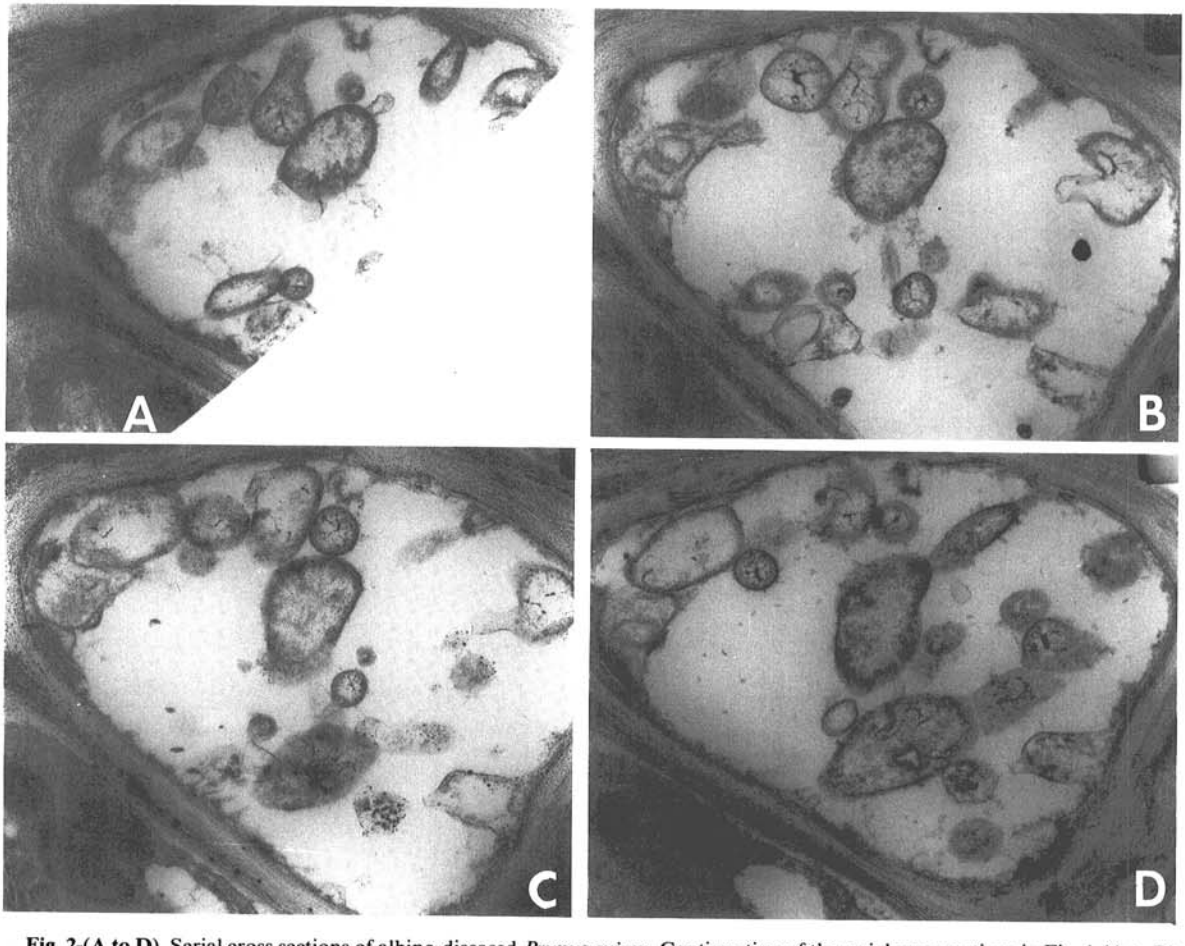

Fig. 2-(A to D). Serial cross sections of albino-diseased *Prunus avium*. Continuation of the serial cross sections in Fig. 1-(A to D). Note that the tubular body visible in Fig. 1-D is not visible in Fig. 2-A. × 22,800 (1mm = 44 nm). See composite stereo pairs of all sections from Fig. 1 and 2 in Fig. 4-A.

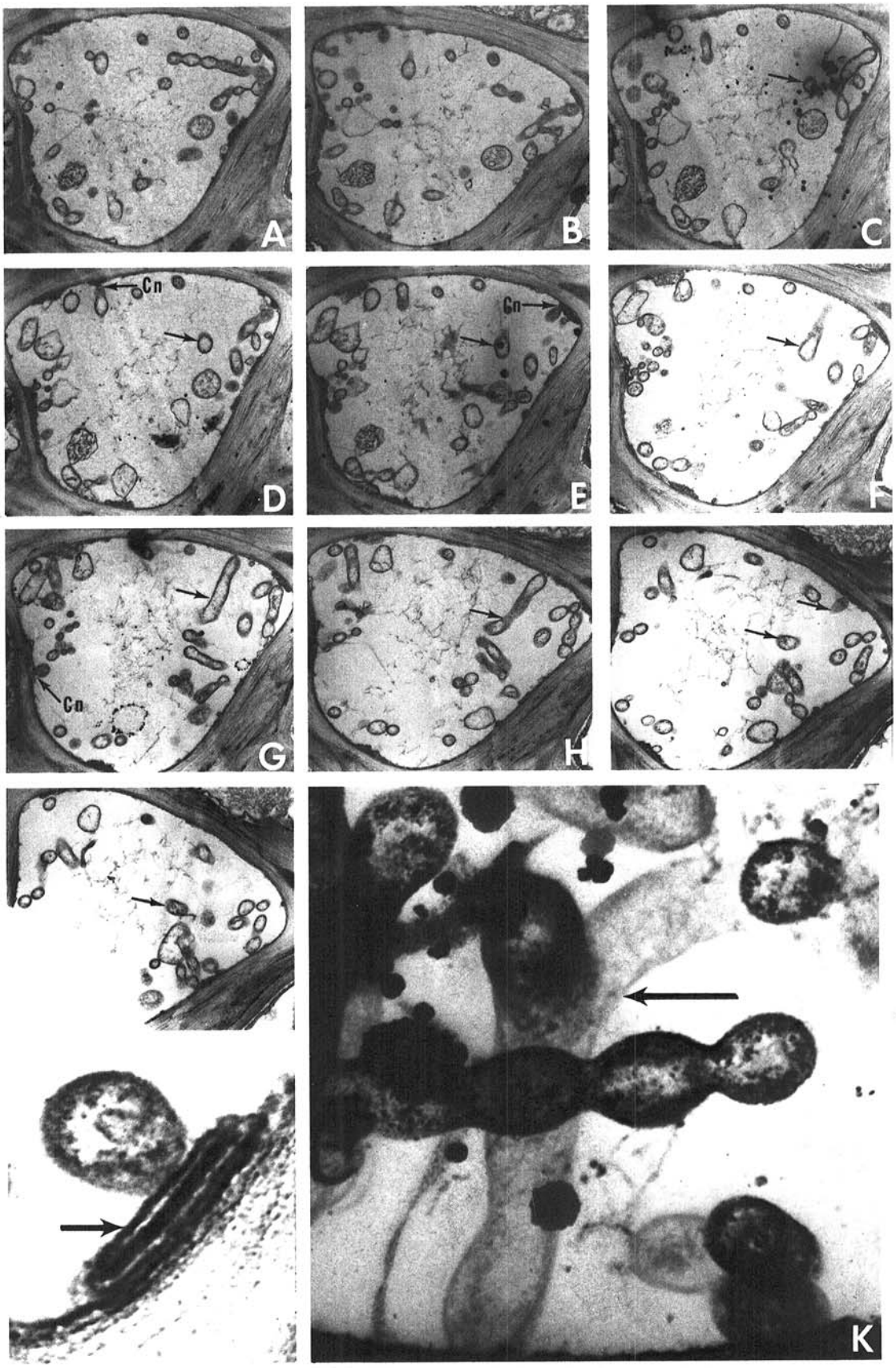

Fig. 3-(A to K). Serial cross sections of mycoplasmalike bodies in sieve tube elements in albino-diseased *Prunus avium*. Note tubular (tu) and spherical (sph) morphology and connections (cn). Figure 3-K is a composite photograph made by stacking transparencies of the serial cross sections A through J. Note that the tubular branched structure in Fig. K (arrow) is made up of structures marked by arrows in section C through J. Fig. 3-L is an enlargement of Fig. 3-A showing stacked endoplasmic reticulum. In sections A through J, the magnification is × 11,300, scale is 1 mm = 88 nm. In K, it is 1 mm = 16 nm. See stereo pairs of these sections in Fig. 4-(B-B to D-D).

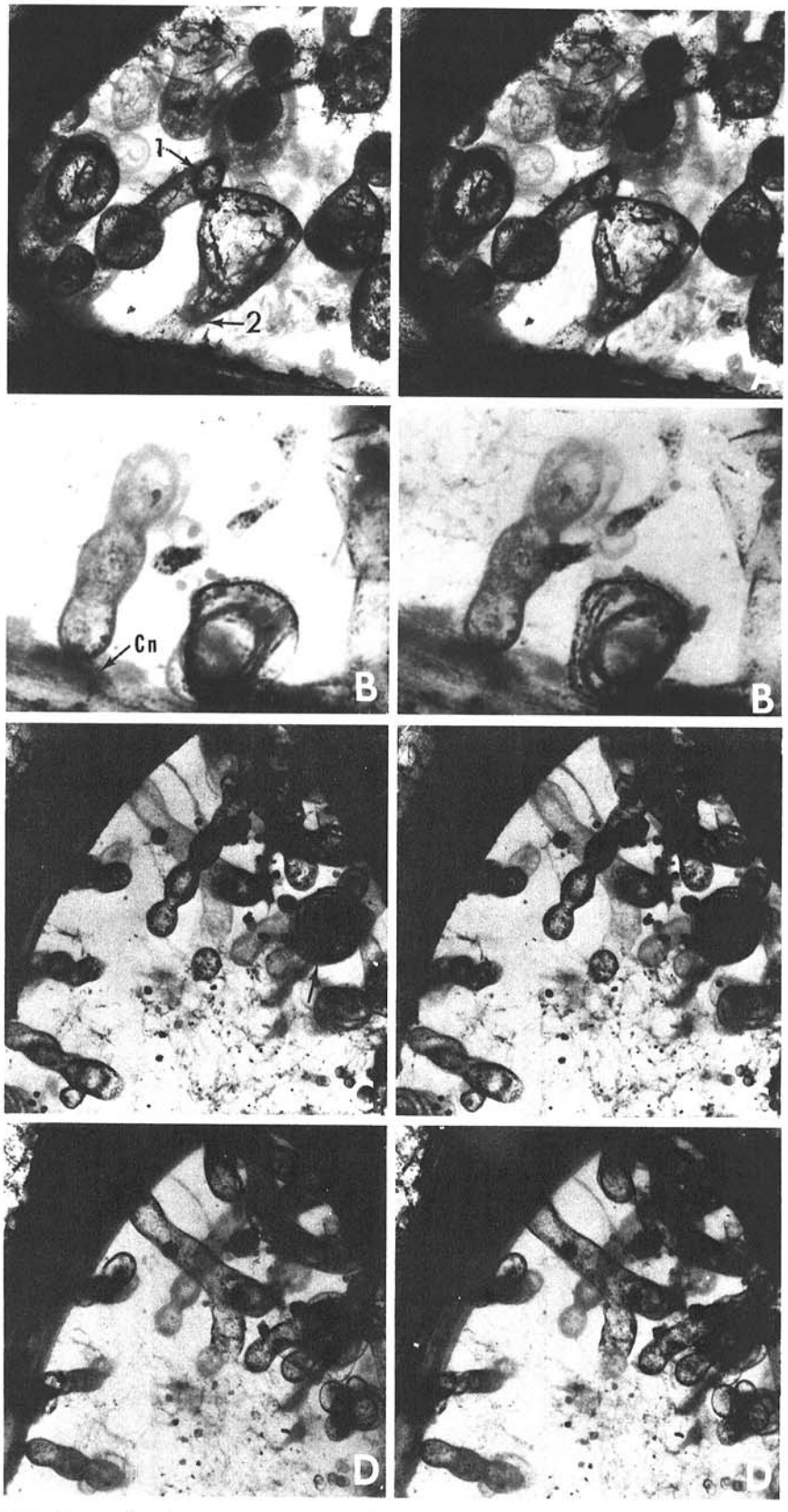

Fig. 4-(A-A to D-D). Stereo pairs of photographs of serial cross sections of mycoplasmalike bodies in sieve tube elements of albino-diseased *Prunus avium* in Fig. 1 and 2. The reader needs a stereo viewer for observation of stereo pairs. **A-A)** Note the connection of the structures numbered 1 and 2 and the three-dimensional structure of the mycoplasmalike bodies. × 20,600 (1 mm = 48 nm). **B-B)** Stereo paired photographs of a structure in the serial sections of Fig. 3-(A-J). Note the connection (cn) and morphology. × 35,500 (1 mm = 28 nm). [(C-C) and (D-D)] Stereo pairs of serial sections in Fig. 3-(A-J) in which **D-D)** is a reciprocal arrangement of the sections in **C-C)**. Note the spherical structures in (C-C) (arrow) is located over several tubular forms and the tubular structure with the constrictions is located above the branched tubular form, whereas in stereo pair D-D they are the opposite. × 22,600 (1 mm = 44 nm).

wall where stacked ER occurs (Fig. 1-D; 3-D, E, F, and 4-B). The connection is small, less than the thickness of one thin-section (60-90 nm), and would most likely not have been observed unless the technique of stacking transparencies of serial sections was used.

Research on *Mycoplasma* sp. associated with animal cells has revealed an extracellular location and the formation of a continuous structure with the cell plasmalemma (1, 7, 13, 14, 20, 22). The attachment of plant MLB to stacked ER may be similar to that found in animal cell/*Mycoplasma* sp. relationships. On the other hand, the presence of the connection raises an interesting point: are the tubular structures that resemble MLB developing from ER or are the MLB attaching to the ER? Not enough information is available to answer this question.

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