Effects of Elevated Temperature on the Ultrastructure of Mycoplasmalike Organisms in Periwinkle

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ABSTRACT

The ultrastructure of mycoplasmalike organisms (MLO) in safflower phyllody-affected periwinkle plants (*Vinca rosea*) kept at 40 C was compared with that in control plants grown at 25 C. MLO in control plants resemble other plant MLO. Small spherical bodies originating by budding from large MLO were observed, as well as numerous free ones, both in sieve cells and within sieve pores. Gradual degradation and disappearance of normal components within the MLO were observed when periwinkle plants were kept at 40 C. Electron-dense material accumulated in clumps near the membrane, and the contents began to disappear after 1 day. No additional changes were observed after 7 days (and up to 50 days) at 40 C, when MLO seem to be almost devoid of ribosomes and other components.

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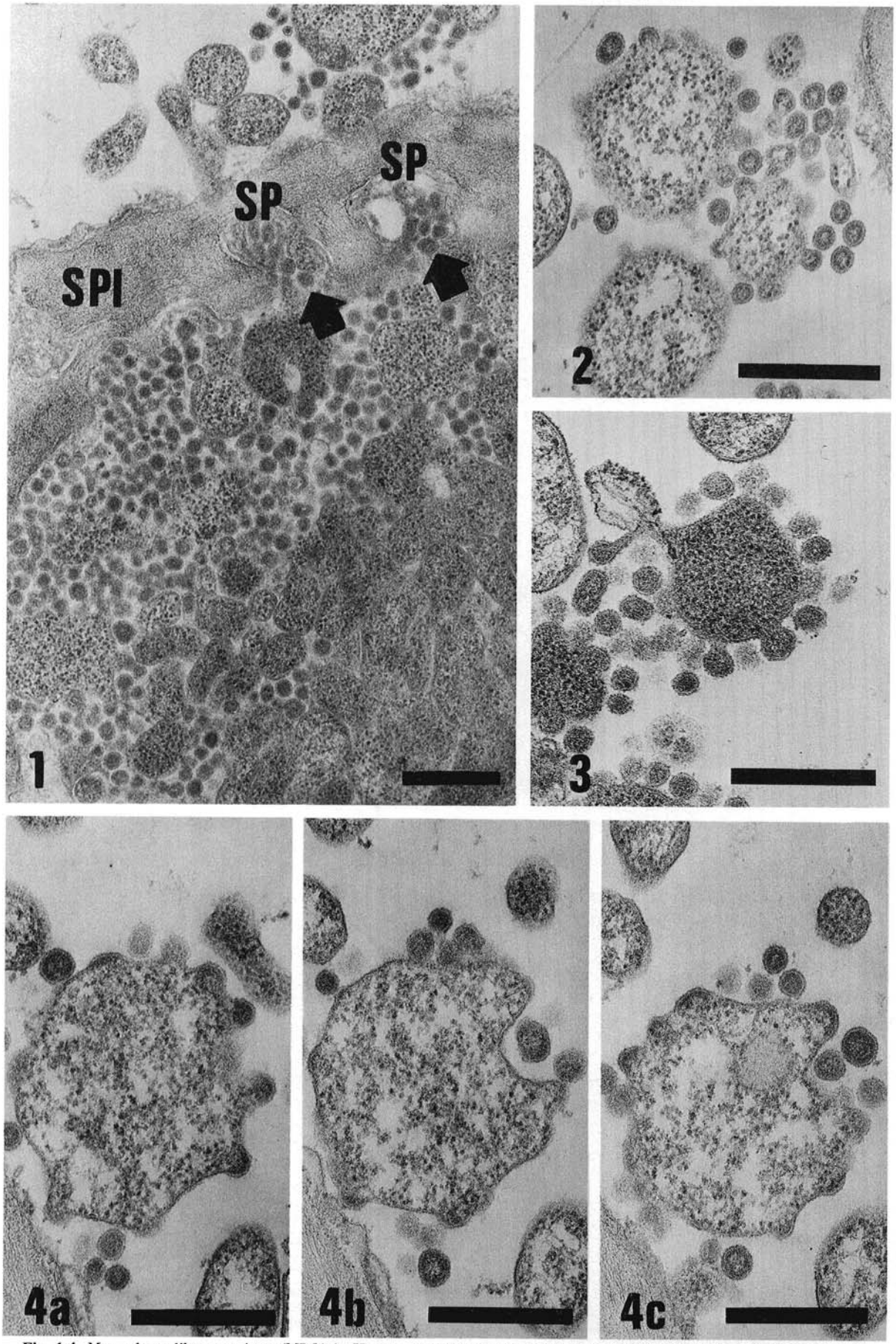

Fig. 1-4. Mycoplasmalike organisms (MLO) in *Vinca rosea* affected with safflower phyllody. **1)** Sieve cell with MLO. Many small spherical bodies in the lower cell and within sieve pores (SP) marked by arrow (SP1 = sieve plate); **2, 3)** Budding forms frequently found in plants with advanced infections. **4-a, b, c)** Serial sections demonstrating budding from large MLO. Bar equals 0.5 μ.

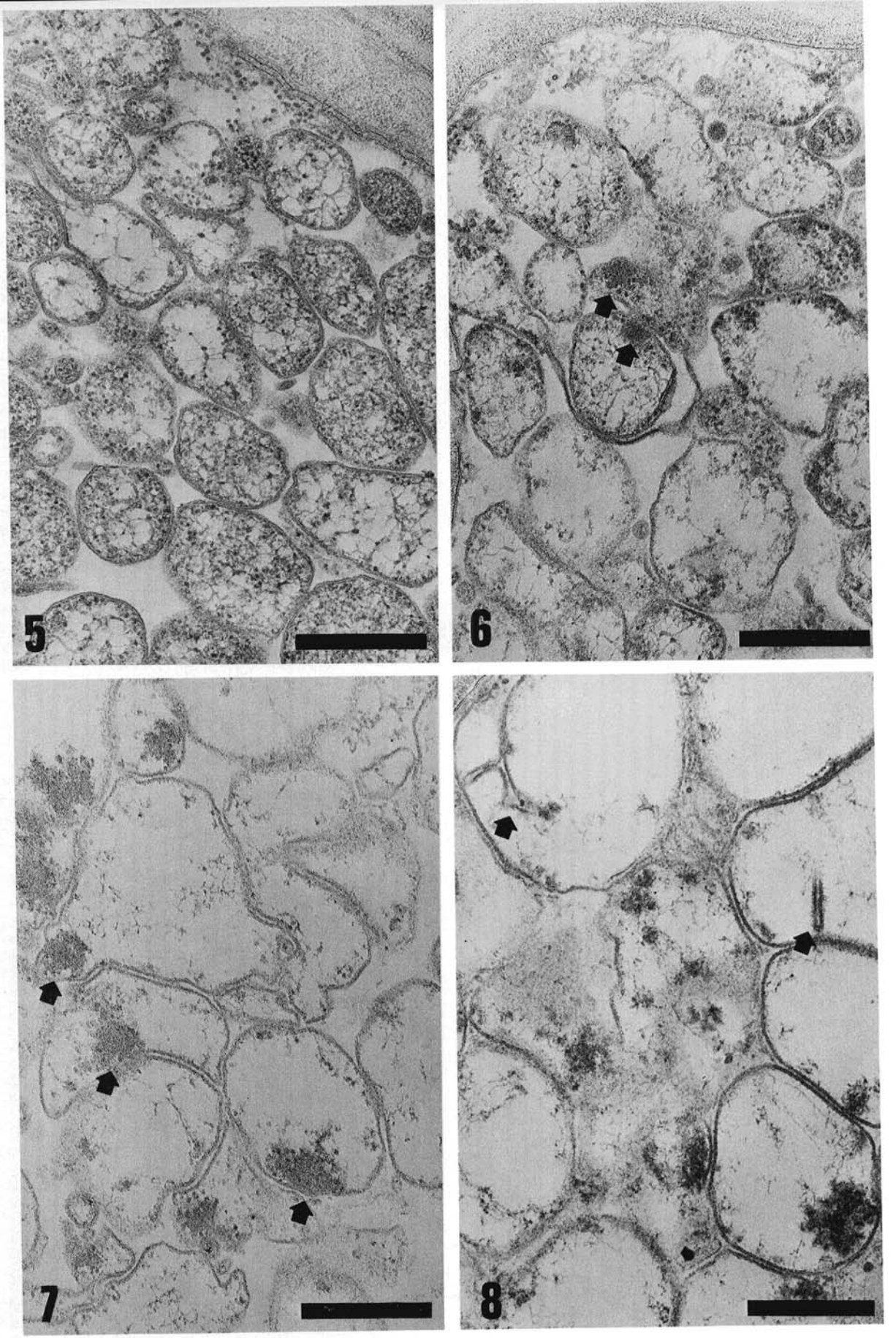

Fig. 5-8. 5) MLO in safflower phyllody-affected *Vinca rosea* maintained at 25 C. **6, 7)** MLO in *V. rosea* kept at 40 C for 1 or 3 days, respectively. Electron-dense clumps near membranes (arrows). **8)** MLO in *V. rosea* kept for 1 week at 40 C. Organisms devoid of normal structures, with typical invaginations (arrows). Bar = 0.5 μ.

Since Kunkel's demonstration of the susceptibility of the aster yellows agent to high temperatures (3), many reports concerning thermotherapy of plants infected with yellows-type diseases have appeared (6). In the last few years, evidence has increased to support the hypothesis that mycoplasmalike organisms (MLO) are the agents of yellows-type diseases which previously were considered to be induced by viruses (1, 5).

We report herein changes in the ultrastructure of MLO, which result finally in their internal degradation, when infected plants are kept at 40 C. These changes are interpreted as additional evidence that MLO have an etiological role in yellows-type diseases.

MATERIALS AND METHODS.—Periwinkle (*Vinca rosea* L.) plants affected with safflower phyllody (2) were obtained from Meir Klein, Faculty of Agriculture, Hebrew University of Jerusalem, Rehovot. Cuttings of these were rooted and grown at 25 C in an insect-free greenhouse. Three-month-old cuttings with severe symptoms (stunting and small yellow leaves) were kept either at 25 C or transferred to a greenhouse chamber at 40 ± 2 C for different periods. Five to 10 samples/treatment were taken for electron microscopy.

For electron microscopy, the middle veins and petioles were cut into 1- to 2-mm segments under cold 5% glutaraldehyde in 0.1 M phosphate buffer, pH 7, and transferred to fresh fixative solution for 2 hr at 4 C. The samples were rinsed and postfixed in 2% (w/v) osmic tetroxide in the same buffer at 4 C. After dehydration through graded ethanol and several passages through propylene oxide, samples were embedded in Epon mixture (4) and polymerized at 60 C for 72 hr. Samples of healthy periwinkles were prepared similarly.

Phloem cells were localized with the aid of a phase-microscope on 2-μ sections, and ultrathin sections (ca. 600 Å) were then cut with glass knives on a LKB Ultrotome III. Sections were stained with aqueous 6% uranyl acetate for 30 min, counterstained with lead citrate for 5 min, and examined with a JEM 7A electron microscope operating at 100 kv.

RESULTS AND DISCUSSION.—MLO, in infected periwinkles maintained at 25 C, were highly pleomorphic, and rounded forms with a diameter of 300-600 nm predominated. The MLO were bounded by a clearly defined "unit membrane" and contained ribosomes and fine strands (2) presumed to be nucleic acid (Fig. 5). Their structure resembled that described for animal and plant mycoplasmas.

Large numbers of small spherical bodies were observed in some of the sieve tubes and within sieve pores in plants with advanced infections, 3-6 months after inoculation (Fig. 1). These bodies ranged between 80 and 120 nm in diam. They were frequently found within sieve pores, probably passing to adjacent sieve cells. They seem to originate mainly by budding from the periphery of pleomorphic large MLO (Fig. 2, 3). Although budding forms have been reported several times (7, 10), their existence has recently been questioned (1). However, sequences of serial sectionings through safflower phyllody-affected periwinkle cells with "budding" MLO (Fig. 4-a, b, c) support the idea that these bodies originated by budding from large MLO.

When infected periwinkles were transferred to a greenhouse chamber at 40 C, changes in MLO ultrastructure, leading to disorganization, became apparent after 1 day (Fig. 6). Electron-dense material starts to accumulate in clumps near the membrane, and the contents begin to disappear. After 3 days at 40 C, the peripheral electron-dense masses are conspicuous and only a few ribosomes and strands remain within the MLO (Fig. 7). After 7 days at 40 C, the MLO seem to be almost devoid of ribosomes and other components. In some MLO, typical invaginations of the membrane were observed (Fig. 5-8).

Additional samples for electron microscopy were taken after 10, 15, 20, 30, 40, and 50 days at 40 C. In some of the sieve tubes, empty MLO were found even after 50 days, but these did not differ in ultrastructure from those observed after 7 days.

The degradation of MLO ultrastructure observed in periwinkle plants kept at 40 C apparently is related to the finding that many yellows diseases are cured by exposure of the affected plants to elevated temperatures. MLO that were devoid of internal structure and had disrupted membranes also were observed in tetracycline-treated plants (8, 9). These considerations provide additional support for the hypothesis that MLO are the causal agents of these diseases. The possibility that heat treatment prevents multiplication of MLO and that the observed changes are due to normal degradative processes, cannot be excluded. However, the degradative changes observed after only 24 hr of heat treatment suggest that at least part of the degradation is heat induced.

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