Ultrastructural changes in cucumber leaves following artificial inoculation with Pseudomonas syringae pv. lachrymans

Tohoku Journal of Agricultural Research
Vol. 31, No. 3, December 1980
Printed in Japan

Ultrastructural changes in cucumber leaves following artificial inoculation with *Pseudomonas syringae* pv. *lachrymans*

Masahiro OZAKI and Susumu YAMANAKA

Laboratory of Plant Pathology, Faculty of Agriculture, Tohoku University, Sendai, Japan

(Received, June 13, 1980)

Summary

Ultrastructural changes in cucumber leaves inoculated with *Pseudomonas syringae* pv. *lachrymans*, a causal bacterium of angular leaf spot of cucumber, were investigated under an electron microscope. The earliest changes in the inoculated leaves were detected on plasma membranes and chloroplasts. Within 24 hr after inoculation some portions of plasma membranes became electron-translucent, swelled and subsequently often became discontinuous. The chloroplasts swelled abnormally within 48 hr. Disorientation of grana lamella, swelling of plastoglobuli and disorganization of the bounding membranes also occurred in swollen chloroplasts. The ultrastructural abnormalities in nuclei, mitochondria and microbodies began to appear 3 days after inoculation or later. In the inoculated leaves, deformed plasmodesmata which were never observed in the uninfected cucumber leaves were occasionally observed in the cell wall. The accumulation of osmiophilic globuli was frequently observed in the cytoplasm of these cells.

Cucumber angular leaf spot, incited by *Pseudomonas syringae* pv. *lachrymans* (Smith & Bryan 1915) Young, Dye & Wilkie 1978, is characterized by the appearance of the water-soaked lesions which are limited by veins. A considerable amount of literature is available on the epidemiological studies of this disease, but little work has been done on the histological and cytological studies of infected leaves. Williams & Keen (1) carried out histological investigation on the water-soaked lesions and reported that the bacteria which entered through stomata in the lower epidermis were largely restricted to the spongy mesophyll by a closely packed layer of cells between the palisade and spongy mesophyll layers. Previously we reported the process of lesion formation on cucumber leaves inoculated with *P. syringae* pv. *lachrymans* by using a scanning electron microscope (2); the cells within the inoculated sites gradually lost turgor pressures and were finally dehydrated. The bacteria which multiplied in the intercellular spaces were often egressed from the stomata.

The present paper describes ultrastructural changes following the development of water-soaked lesion.

Materials and Methods

Plant, Bacterium and Inoculation

Cucumber (*Cucumis sativus L.*) plants, a cultivar Yamatosanjyaku which is susceptible to angular leaf spot, were used. Plants were grown in 5-inch pots in a glasshouse and used at the 3rd leaf stage.

An isolate No. 7417 of *P. syringae* pv. *lachrymans* was used. The isolate was grown on Wakimoto's medium (15 g sucrose, 5 g peptone, 0.5 g calcium nitrate, 2 g sodium phosphate, dibasic, per liter of the broth from 300 g of boiled potatoes, pH 7.0) for 48 hr at 27°C. For inoculum preparation, the bacteria were collected by suspending cells from an agar slant in distilled water, centrifuged and resuspended in fresh distilled water. Bacterial concentrations were adjusted photometrically to absorbance of 0.1 at 620 nm (ca. 10^8 cells/ml).

The bacteria were inoculated in the lower surface of cucumber leaves with a spray gun as described previously (2). Inoculated plants were kept in a moist chamber held at 25°C for 48 hr, and then they were returned to the vinyl-box in a glasshouse.

Preparation for Electron Microscopy

Pieces of leaf (ca. 2 mm^2) were cut from the inoculated areas at 0, 1, 2, 3, 4, 6 and 8 days after inoculation. Excised tissues were fixed in 2 per cent osmium tetroxide for 4 hr at 4°C. After washing, the material was dehydrated in alcohol, followed by propylene oxide and embedded in Epon–812 according to usual procedures. Ultrathin sections were cut with an ultramicrotome (Porter-Blum, MT–2B) equipped with a glass knife, stained with aqueous uranyl acetate and lead citrate, and observed with a JEM 100B electron microscope (Japan Electric Optics Laboratory Co. Ltd.).

Results

Symptoms

The areas of cucumber leaves inoculated by spraying forcibly bacterial suspension with a spray gun, were temporarily water-soaked, but the water-soaking disappeared within 2 hr. As an early symptom, small water-soaked lesions in the infected tissue first appeared at 24 hr after inoculation, and the lesions enlarged during the following 24 hr. Initially such lesion areas appeared to be water-rich, but they gradually dehydrated, decreased in size and finally the tissue containing the dehydrated cells became very thin. Next yellow halos appeared around the lesions. Subsequently, the matured lesions turned white or whitish brown and were occasionally encircled by narrow brown bands.

Ultrastructural Changes in Infected Leaves

Ultrastructural changes in spongy and palisade mesophyll cells in the infected tissue were examined in comparison with healthy leaves.

Fine structures of healthy cucumber cells are shown in Figs. 1–2. Plasma membrane was observed near the cell wall. In the chloroplasts, grana lamella, starch grains and a few plastoglobuli were observed. The two closely appressed envelopes of limiting membrane in the chloroplast were clearly visible. Mitochondria with tubular cristae and microbodies were usually seen near the chloroplasts. Normal nuclei were often observed in the proximity of the cell wall. Also the cell damage was not discernible in the tissues inoculated with distilled water only.

On the other hand, at the early stage of lesion development in the inoculated leaves, marked structural changes were observed in plasma membranes and chloroplasts. During the first 24 hr after inoculation plasma membranes frequently separated from the cell wall, became partially electron-translucent and swelled two- or three-fold in width when compared with that of uninfected leaves (Figs. 3–4). Furthermore, the unit-membrane structure of these portions became obscure. Some parts of such plasma membranes often became discontinuous for the next 24 hr and later (Fig. 5).

The chloroplasts slightly swelled in the first 24 hr, but no changes of their internal structures were noticed. The chloroplasts abnormally enlarged in the next 24 hr and the stroma area became electron-transparent (Fig. 6). The plastoglobuli in the swollen chloroplasts also enlarged in size. Three or four days after inoculation the shapes of chloroplasts became irregular and the bounding membranes of chloroplasts occasionally became discontinuous (Fig. 10). In such chloroplasts, plastoglobuli, starch grains and the remnants of grana lamella were conspicuous, but more detailed structures within the chloroplasts were not discernible. Eight days after inoculation the chloroplasts which seemed to have lost the functions were aggregated because of the agglutination of cytoplasm, and only the stach grains were visible (Fig. 13).

Ultrastructural changes of nuclei, mitochondria and microbodies were observed 3 days after inoculation and later. The matrix of nuclei of the cells in the inoculated areas became slightly electron-lucent and later the electron-density severely decreased accompanying the agglutination of chromatin (Fig. 7). Mitochondria were often irregularly deformed, and their membranes were disrupted at several parts (Figs. 8–9). The microbodies occasionally contained electron-dense nucleoids which were rarely observed in uninfected cucumber leaves (Figs. 8–9).

In the cells from the center of matured lesions, plasmodesmata were observed as circular or elliptical structures in cross sectioning in the cell wall (Fig. 11). These irregular shapes of plasmodesmata were not observed in the wall of uninfected mesophyll cells. In uninfected cells plasmodesmata were observed as tubular structure across the cell wall, whose shapes were similar to those seen in vascular

bundle cells. The cell walls which contained such malformed plasmodesmata lacked lamellar structure of cellulose layer. Also these plasmodesmata were predominantly observed in the cell wall of severely degenerated mesophyll cells. In those cells osmiophilic globuli were also found in the cytoplasm (Fig. 12).

Discussion

The earliest ultrastructural changes in cucumber mesophyll cells inoculated with *P. syringae* pv. *lachrymans* were observed in plasma membranes and chloroplasts. As shown in Figs. 3–5, the plasma membranes occasionally swelled, disorganized and became discontinuous. Similar swelling or disorganization of plasma membranes has also been observed as initial abnormalities in other diseases (3). Williams & Keen (4) reported that the leakage of electrolytes from the cucumber leaves infected with *P. syringae* pv. *lachrymans* decreased for 24 hr after inoculation, but later rapidly increased following the enlargement of a water-soaked lesion. We observed a similar phenomenon in the infected cucumber leaves, although the time course of leakage of electrolytes differed slightly*. The disappearance of unit-membrane structure on plasma membrane observed 48 hr after inoculation may be responsible for leakage of electrolytes from the infected cells.

The fine structural changes of the chloroplasts appeared to be basically similar to the cases of many other diseases (5–8). Plastoglobuli in the chloroplasts of the infected leaves enlarged 3–5 fold in diameter in comparison with those of uninfected leaves (Fig. 10). It is well known that the plastoglobuli in chloroplasts of leaves infected with virus enlarged in size and increased in number (9). Thus, these changes were not specific reactions to the bacterial infection. Also it has been clearly proven that the size and number of plastoglobuli in chloroplasts increased in concomitant with leaf senescence (10, 11).

Circular or elliptical shapes of plasmodesmata were occasionally observed in the cell wall of the infected mesophyll cells (Fig. 11). This probably resulted from the denaturation of the cell wall due to bacterial infection.

The ultrastructural changes in nuclei, mitochondria and microbodies were observed beginning with the 3rd day after inoculation and later. These changes also are not specific to this disease. Therefore, it is suggested that the changes of these organelles are mainly due to the response resulting from cell death.

The microbodies of the infected leaves sometimes contained dense nucleoids which are rarely observed in uninfected cucumber cells. Similar results were reported by Goodman & Plurad (3) in tobacco leaves infected with *Pseudomonas syringae* pv. *pisi*.

In the later stage of infection, osmiophilic globuli were often observed in the cytoplasm (Fig. 12). Similar globuli were often observed in other diseased plant

* unpublished experiments

tissues (12, 13), and these bodies seemed to be lipid globuli.

P. syringae pv. *lachrymans* in the infected tissues, as well as most of phytopathogenic bacteria, was consistently observed only in the intercellular spaces and never noted in the host cells. Therefore, several fine structural changes caused by bacterial infection are probably due to phytotoxic metabolites produced by the bacterium in the intercellular spaces.

References

1) Williams, P.H., and Keen, N.T., *Phytopathology*, **57**, 254–256 (1967)
2) Yamanaka, S., Ozaki, M., and Kato, S., *Tohoku J. Res.*, **30**, 135–141 (1980)
3) Goodman, R.N., and Plurad, S.B., *Physiol. Plant Pathol.*, **1**, 11–15 (1971)
4) Williams, P.H., and Keen, N.T., *Phytopathology*, **57**, 1378–1385 (1967)
5) Arnott, H.J., and Smith, K.M., *J. Ultrastru. Res.*, **19**, 173–195 (1967)
6) Coffey, M.D., Palevitz, B.A., and Allen, P.J., *Can. J. Botany*, **50**, 1485–1492 (1972)
7) Navratil, S., and Boyer, M.G., *Can. J. Forest Res.*, **2**, 308–312 (1972)
8) Delon, R., Kiffer, E., and Mangenot, F., *Can. J. Botany*, **55**, 2463–2470 (1977)
9) Tomlinson, J.A., and Webb, M.J.W., *Physiol. Plant Pathol.*, **12**, 13–18 (1978)
10) Greenwood, A.D., Leech, R.M., and Williams, J.P., *Biochim. Biophys. Acta*, **78**, 148–162 (1963)
11) Butler, R.D., *J. Exptl. Botany*, **18**, 535–543 (1967)
12) Lasarovits, G., and Higgins, V.J., *Can. J. Botany*, **54**, 235–249 (1976)
13) Kunoh, H., Kohno, M., Tashiro, S., and Ishizaki, H., *Can. J. Botany*, **57**, 2501–2508 (1979)

Abbreviations used in Figures

B; bacterium, C; chloroplast, CW; cell wall, IS; intercellular space, M; mitochondrion, MB; microbody, N; nucleus, OG; osmiophilic globulus, PD; plasmodesma, PG; plastoglobulus, PM; plasma membrane, S; starch grain, VA; vacuole.

Bars represent 0.5 μm except in Fig. 13.

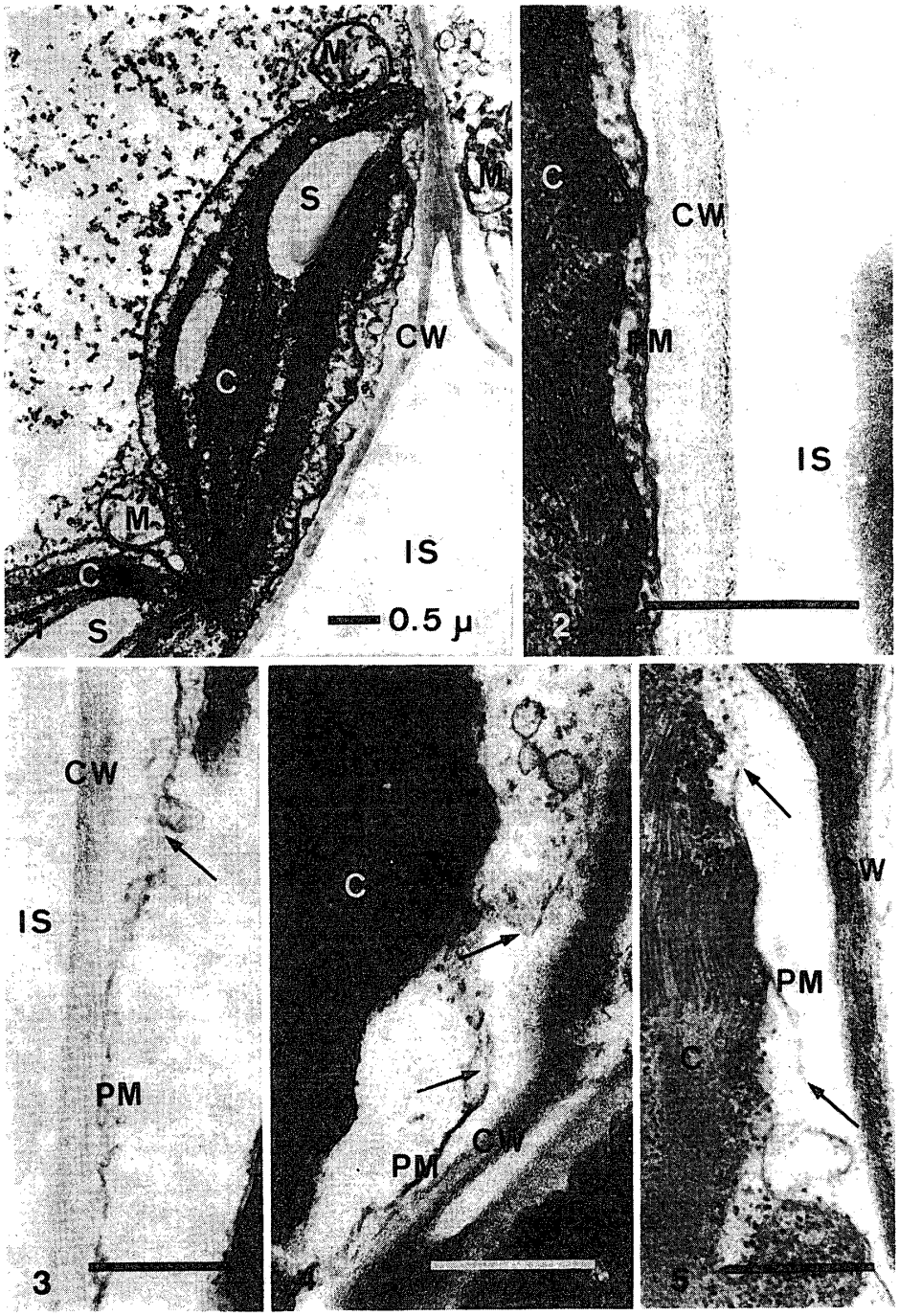

FIG. 1. Mesophyll cells of a healthy cucumber leaf. Cell organelles such as mitochondria and chloroplast show normal structure.

FIG. 2. A plasma membrane observed near the cell wall of a healthy cucumber mesophyll cell.

FIGS. 3, 4. Some portions of plasma membranes enlarged in width (arrows). At such sites the unit-membrane structure was obscure. 1-day infection.

FIG. 5. Some portions of plasma membrane became discontinuous (arrow). 2-day infection

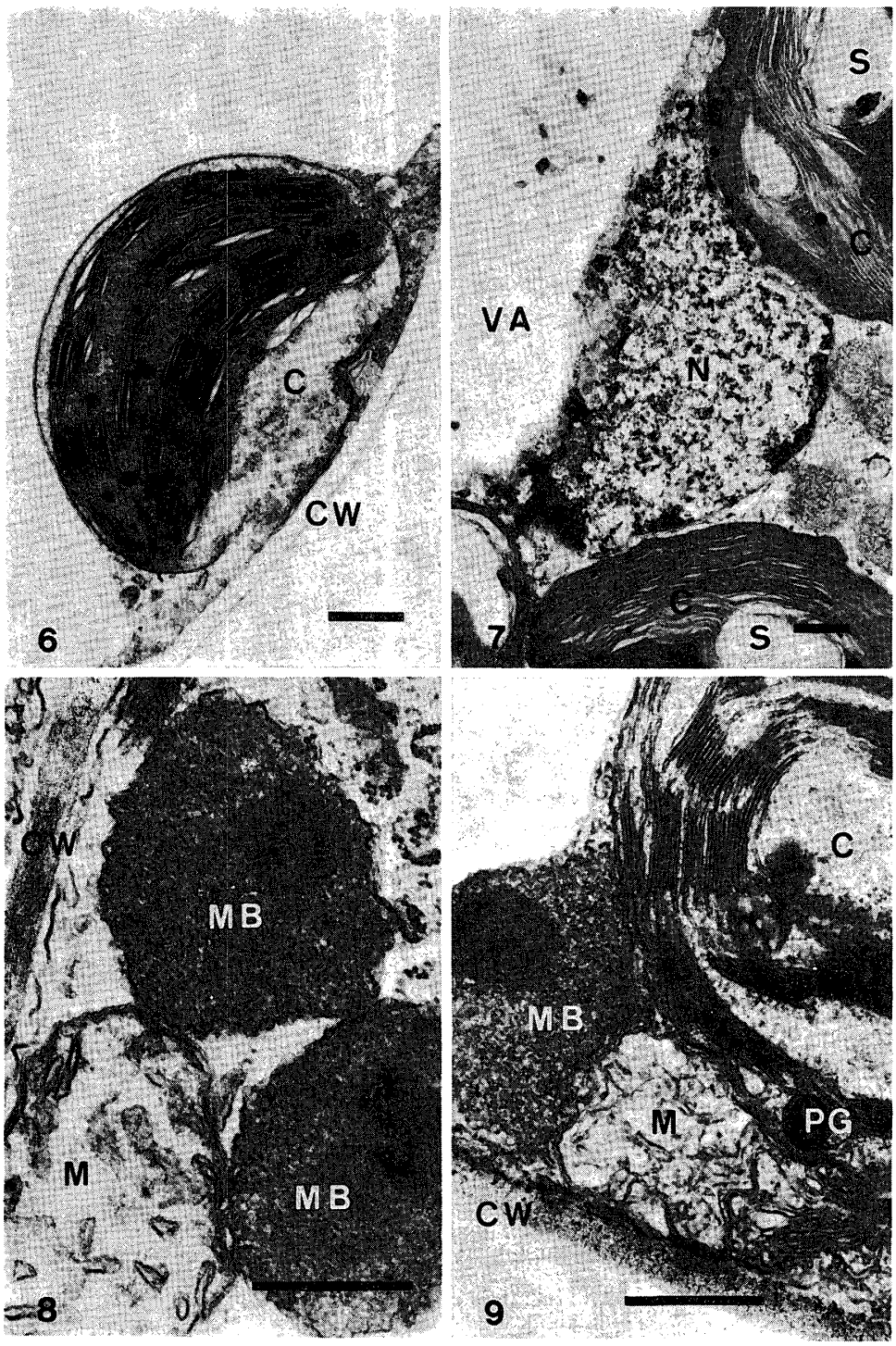

FIG. 6. The chloroplast abnormally swelled. Disorientation of grana lamella and swelling of plastoglobuli were noted. 2-day infection.

FIG. 7. The matrix of nucleus became electron-translucent and chromatin was agglutinated. 3-day infection.

FIG. 8. Microbodies within severely infected cells occasionally contained dense nucleoids. 4-day infection.

FIG. 9. Mitochondrion was severely deformed and degenerated. 6-day infection.

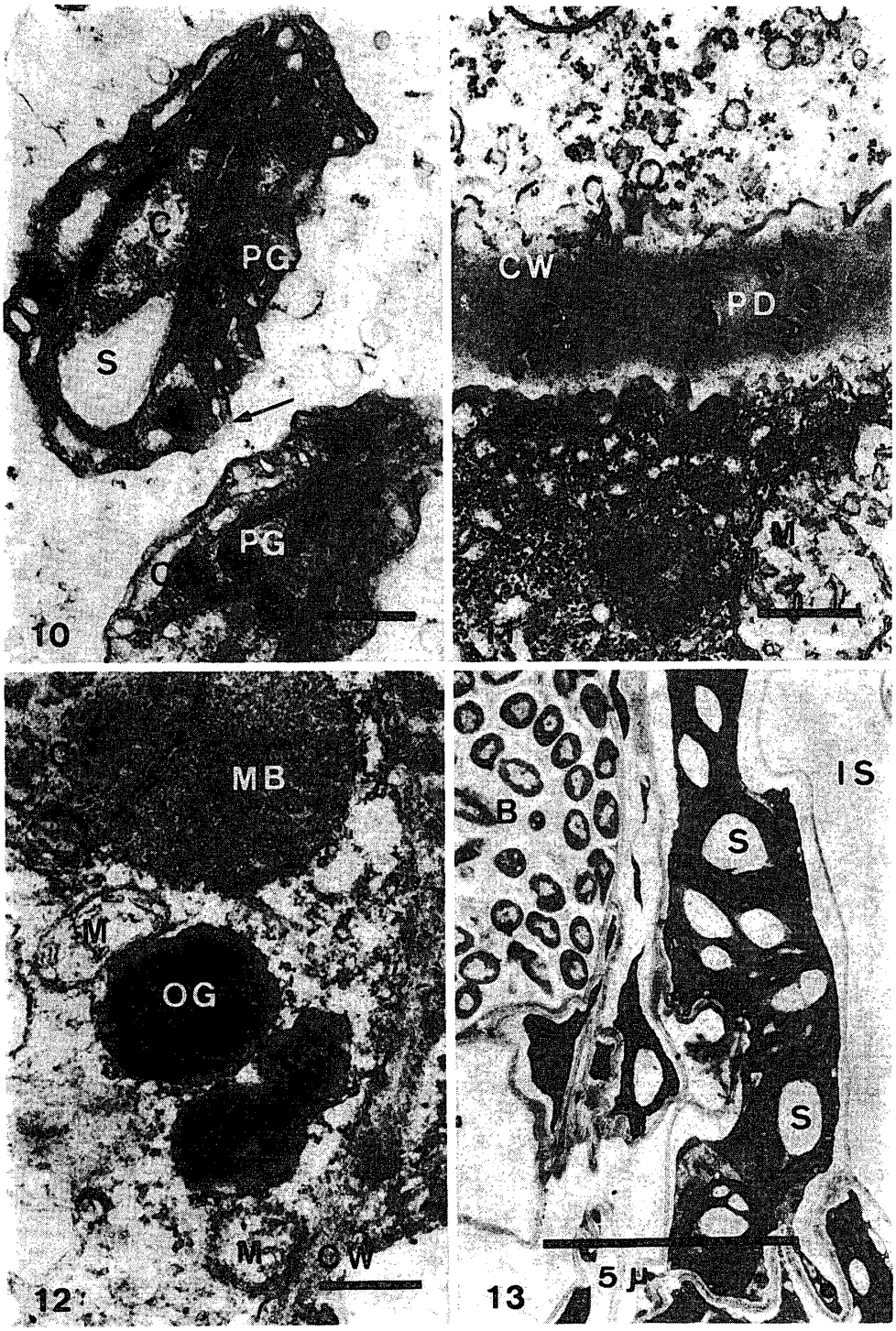

FIG. 10. The bounding membranes of chloroplasts became less discernible or discontinuous (arrow). Plastoglobuli heavily enlarged. 6-day infection.

FIG. 11. In severely infected cells many irregular shapes of plasmodesmata were observed in the cell wall. 6-day infection.

FIG. 12. Osmiophilic globuli accumulated in the cytoplasm of a collapsed cell. 6-day infection.

FIG. 13. At late stage of lesion formation the internal structures of the chloroplasts despite presence of starch grains were obscure because of the compact agglutination of cytoplasm. 8-day infection. Bar represents 5 μm.