Ultrastructure of Lesions Produced in Leaves of *Beta vulgaris* by Cercosporin, a Toxin from *Cercospora beticola*

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ABSTRACT

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Lesions induced in leaves of *Beta vulgaris* 5 days after application of cercosporin, one of two or more toxic metabolites produced by the fungus *Cercospora beticola*, were examined by electron microscopy and compared with a published report of lesions incited by the fungus. Both types of lesions showed large amounts of granular, electron-dense intercellular material, especially in lesion margins; cells with electron-dense cytoplasmic ground substance and cell-wall appositions; and the loss of cell membranes, especially the chloroplast-bounding membranes and tonoplast. Both lesion types contained necrotic cells that had collapsed during the degenerative sequence. Necrotic cytoplasm within these cells contained starch grains, remnants of the chloroplast lamellar membranes, and sometimes areas that had once held crystalline material. Unlike fungus-induced lesions, however, cercosporin-induced lesions did not have electron-dense bodies in vacuoles and necrotic remnants, lacked a well-defined boundary zone with generalized wall thickenings, and usually had no increases in the size or number of plastoglobuli.

Additional key words: Cercospora beticola toxin (CBT), nonhost-specific toxin.

Although research on cercosporin and other metabolites of the deuteromycete fungus *Cercospora* has been carried out for at least 28 yr, reports of these studies are widely scattered in the scientific literature and have never been fully reviewed. This situation has resulted in misunderstandings and erroneous claims by some authors. For instance, Schlösser did not work with cercosporin, as reported in some papers (35,37,63), and Fajola (18) was not, as he believed, the first to isolate and partially characterize cercosporin. To bring together and clarify the literature on the metabolites of *Cercospora*, we offer a brief review.

Red pigmentation in cultures of *Cercospora* was noted as early as 1928 by Schmidt (58) in *C. beticola* Sacc. Other early reports (61,64) also included observations of the pigment. In 1953, Deutschmann (17) isolated and crystallized the red pigment from *C. kikuchii* and recognized its presence in infected soybeans. Frandsen (22) studied the formation of both red and yellow pigments by *C. beticola* in culture. Kuyama and Tamura (30) isolated and purified the red pigment from *C. kikuchii* and gave it the trivial name "cercosporin." Further work determined the physical and chemical properties of cercosporin and the fundamentals of its structure (29,31).

Venkataramani (63) in 1967 isolated cercosporin from *C. personata* and from infected *Arachis hypogaea* L. and first demonstrated its toxicity to a host plant. Balis and Payne (8) isolated cercosporin from *C. beticola* cultures and, by applying it exogenously to leaves, showed its toxicity to sugar beet (*Beta vulgaris* L.). Mumma, Lukezic, and Kelly (43) described cercosporin from *C. hayi*. The structure of cercosporin, a perylene quinone, was independently elucidated by Lousberg et al (32) and Yamazaki and Ogawa (66).

Factors influencing cercosporin production in culture have been reported in several publications (8,18,38,40,41,43), and biosynthesis of the toxin has been examined (45). Toxicity of cercosporin or cercosporin derivatives to other organisms, including bacteria, algae, yeasts, higher plants, and mice (13,30,60), and the nature of its photodynamic effects (13,34,67), have been discussed.

Recently, Lynch and Geoghegan (35) reported different amounts of cercosporin production in seven of 12 species of *Cercospora*. Assante et al (6) surveyed 61 species, including six isolates of *C. beticola*, for production of cercosporin and other secondary

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metabolites; 23 of these species and, notably, all but one of the isolates of *C. beticola* produced cercosporin. Fajola (18) reported cercosporin production in nine of 17 species he studied.

Pseudocercosporella capsellae also has been reported to produce cercosporin in vitro (50). Furthermore, the generic relationships of some cercosporin-producing species of *Cercospora* have been examined and revised (15,16). Thus, the ability to produce cercosporin is scattered throughout the genus *Cercospora*, as well as a complex of related genera. This conclusion modifies earlier implications that cercosporin production is limited to the genus *Cercospora* (36) or is invariably displayed by all species properly placed in the genus (18,19).

The yellow pigment studied by Frandsen (22) was isolated by Schlösser (52) from cultures of *C. beticola* and shown to be toxic to bacteria and higher plants. Schlösser and coworkers examined some chemical and biological properties of this toxin (53,55–57) but never discussed its relationship to the red pigment (cercosporin). Balis and Payne (8) also isolated a toxic yellow material (in addition to cercosporin) from cultures of *C. beticola*; they thought that the yellow material Schlösser isolated was a mixture of cercosporin and their yellow triglyceride fraction, because the isolation techniques Schlösser used would not have separated the two materials. However, Assante et al (6) recently reexamined one of Schlösser's isolates of *C. beticola* and found that it does not produce cercosporin, rather a yellow toxin that, by partial characterization, is not the triglyceride material of Balis and Payne. Thus, the yellow toxin first called GF (for Gelben Fraktion) (52) and now called CBT (for *Cercospora beticola* toxin) (6,39,54) appears to be separate and distinct from cercosporin. Preliminary trials in our laboratory (S. S. Martin, *unpublished*) indicated that the "yellow fraction" sensu Balis and Payne (obtained by their methods from the same *C. beticola* isolate) can be resolved by column chromatography into an oily, triglyceridelike portion and a material with the ultraviolet absorption characteristics of CBT (6). One other *Cercospora* species, *C. bertoreae*, has been reported to produce CBT (6). Other metabolites of *Cercospora* spp. are being investigated (4,5,7,12).

Cercosporin, Balis and Payne's yellow toxin, and CBT all produce necrotic lesions when applied externally to sugar beet leaves (8,52), but such lesions have not been examined to determine the cytological effects of the toxins. This study was initiated to determine the ultrastructural effects of cercosporin in forming necrotic lesions in sugar beet leaf tissue and to compare these effects with those in lesions produced by the fungus (21,59) to help clarify the role of cercosporin in this host-pathogen interaction.

MATERIALS AND METHODS

Production and extraction of cercosporin. Single-spore isolates of *C. beticola* were cultured on potato-dextrose agar under fluorescent light at 15 C. After 10–14 days, both the agar and the mycelial mat were extracted with ethyl acetate. The extract was filtered, washed three times with water, dried through anhydrous sodium sulfate, and rotary-evaporated at less than 40 C to dryness. The residue was taken up in chloroform and adsorbed onto phosphate-treated silica gel (8), which was then dried under vacuum at 30 C until the material flowed freely. This material was added to the top of a column (1 × 50 cm) packed with phosphate-treated silica gel. The column was eluted with ethyl acetate-cyclohexane (1:1, v/v), and fractions were collected.

The deep red, late-eluting cercosporin fractions were combined, evaporated to dryness, and again column-chromatographed similarly. These cercosporin fractions were combined and evaporated to dryness. The product was crystallized twice from ethanol-water at 4 C. Ultraviolet and visible absorption maxima in ethanol were 224, 260sh, 275, 325, 470, 526sh, and 564 nm. The infrared spectrum (KBr window) included major bands at 3400, 2940, 1619 (quinone carbonyl), 1586, 1555, 1455, 1428, 1315, 1268, 1170, 1145, 1113, 1075, 1055, 1017, 976, 938, 920, and 850 cm^{-1}. These data correspond closely to published data for cerocosporin (8,30,32,66). One portion of the purified material was diluted in absolute ethanol to a cercosporin concentration of 0.5 mg/ml.

Application of cercosporin. *B. vulgaris* 'SP 5822-0' plants with an intermediate level of field resistance to *C. beticola* leaf spot disease were grown from seed in steamed soil in a greenhouse. Cercosporin solution was applied with a microsyringe in 1-μl droplets to the adaxial surface, away from major veins, on mature leaf blades. Similar droplets of ethanol were applied to the other half of the same leaf blades. Leaf tissue at the centers of the ethanol control droplets and of some of the cercosporin droplets was pricked once with the syringe needle to facilitate entry of materials into the leaf. Immediately after treatment, plants were placed in a mist chamber for 5 days at 30 C, 90–100% relative humidity, and 16 hr of fluorescent light (5,600 lux) daily.

Tissue sampling and processing. Five days after treatment, leaf tissue containing chlorotic or necrotic depressed areas at the sites of cercosporin application and ethanol control areas was removed and processed for light and electron microscopy. The acrolein-glutaraldehyde and osmium tetroxide fixation, dehydration, embedding procedures, and staining of monitor sections with azure B were described previously (59). Paradermally cut thin sections were stained with Millonig's lead (42) or with uranyl acetate and Millonig's lead.

RESULTS

Ethanol control lesions. No macroscopic or microscopic lesions were found where ethanol droplets had been applied unless the site had been physically wounded at the time of application, in which case a small necrotic area surrounded the wound. Tissue immediately surrounding the necrotic area appeared healthy; that is, comparable to untreated control leaf tissue (59).

Examination of the damaged area of ethanol control lesions with the electron microscope showed collapsed cells containing electron-dense necrotic cytoplasm at the wound site. No "crystallized" areas were seen in the cytoplasmic remains. Degenerative changes were seen in two to three layers of cells bordering the necrotic area. Cells most peripheral to this region appeared healthy except for a slight tendency of the organelles to be grouped or stacked together.

In moderately degenerated cells, fragmentation of the tonoplast was sometimes apparent, as was loss of the ground substance of organelles, especially the chloroplasts and nuclei. Although organelle-bounding membranes generally were intact, nuclear and chloroplast shapes often were highly irregular. Some vacuolation of the cytoplasm also was noted in these cells and in those with more extensive degenerative changes. Cells that bordered the necrotic area of the lesion were extensively collapsed and degenerated. The plasmalemma was recognizable in localized areas only. Organelle-bounding membranes, when present, appeared extremely electron-dense and thickened.

Cell walls appeared intact and relatively unaltered throughout these lesions. No cell-wall appositions were found, although electron-dense granular, fibrillar, or membranous material was sometimes seen in the space that developed between the plasmalemma and cell wall. Very little intercellular material was found in the intercellular spaces or associated with cell walls. Plastoglobuli and starch contents of the chloroplasts never appeared to be altered relative to those of untreated control cells.

Cercosporin-induced lesions. Lesions with necrotic centers surrounded by somewhat chlorotic halos that graded into apparently healthy green tissue at the periphery were observed 5 days after cercosporin was applied. In general, these lesions resembled, in both size and appearance, the 10- to 13-day leaf spots induced by *C. beticola* under greenhouse conditions (although the cercosporin-induced lesions were often more irregular in shape).

Ultrastructurally, the centers of cercosporin lesions consisted of collapsed cells filled with electron-dense cytoplasmic remains. Several layers of cells in varying stages of collapse and necrosis surrounded this necrotic center in the area corresponding to the visually chlorotic halo. Usually, more severely affected cells were found closer to the necrotic area and less damaged cells were found toward the edge, but cells with more or less degeneration could be found interspersed throughout the lesion area.

Near the outer margin of the chlorotic area of the lesion, cells in which the ground substance of the cytoplasm was extremely electron-dense were found interspersed with cells in which the ground cytoplasm showed no alteration from that of healthy control cells (Fig. 1). Cells of both types in this area were only slightly if at all collapsed but had localized or more extensive areas where the plasmalemma had separated from the cell wall (Fig. 1). In these areas of separation, vesicular, membranous, or granular materials often were found (Fig. 1), but no appositional material had been deposited onto cell walls. Small to large vacuoles often were found in the cytoplasm (Fig. 1), and smaller vesicles sometimes were common in cells with unaltered ground cytoplasm. Structural modifications occasionally were seen in organelles of cells with apparently healthy ground cytoplasm. Some mitochondria showed alterations in internal structure; organelle-bounding membranes, especially of the mitochondria and chloroplasts, appeared to adhere to each other or coalesce (Figs. 2 and 3). Plastoglobuli occasionally were larger than those in chloroplasts of untreated tissue, but no increases in starch contents of chloroplasts were evident.

Some structural modifications of cell organelles were seen in cells with the electron-dense ground cytoplasm. Mitochondria frequently lacked normal internal structure, instead having greatly reduced and indistinct cristae (Fig. 1). The condensed chloroplast matrix obscured the lamellar membranes (Fig. 4). Ribosomes, endoplasmic reticulum (ER), and organelle-bounding membranes were barely visible in the electron-dense cytoplasm, but where they could be resolved, they appeared intact (Figs. 1 and 4). Granular, electron-dense material, sometimes in large amounts, often was found in the intercellular spaces in this area of the lesion (Figs. 1 and 4). This material sometimes followed the contours of a cell wall or filled the angles between adjacent cells (Fig. 1). Cell walls in contact with this material did not appear structurally different from cell walls in untreated tissue or in tissue beyond the lesion margin, where this material was absent.

In cells of the area centripetal to that just described, additional degenerative changes were seen (Figs. 5–9). Moderate amounts of electron-dense intercellular material were associated with the cells of this region but rarely occluded the intercellular spaces (Fig. 5). Irregular folds or shallow loops often were seen in the cell walls, and overall, cells appeared to be collapsing (Fig. 5). The cell wall and protoplast also separated; the resulting electron-lucent space sometimes contained granular or membranous material (Fig. 5). In addition, well-formed cell-wall appositions sometimes were found in these cells (Fig. 6). Although the plasmalemma and tonoplast appeared intact in most cells, occasional disruptions in continuity, especially of the tonoplast, were noted (Fig. 6), and both membranes frequently had highly irregular contours (Fig. 6). Cell organelles all were recognizable despite ultrastructural changes in some. Nuclear shape often was irregular (Fig. 9); chromatin was condensed peripherally (Fig. 7) or scattered throughout the central area of the nucleoplasm, as shown in a more degenerated cell (Fig. 10). Chloroplasts were somewhat swollen, sometimes having large outpocketings of stroma and disruptions of the bounding membrane (Fig. 5). Plastoglobuli and starch generally were present, but not in greater than usual amounts.

Several changes were noted in the mitochondria of moderately degenerated cells (Figs. 5, 8, and 9). In some mitochondria, poorly defined cristae in an electron-dense matrix were seen (Fig. 5). The bounding membranes of some mitochondria were disrupted (Fig. 8), but in a few other cells, mitochondria were swollen and almost devoid of ground material (Fig. 9).

Microbodies rarely were seen. The ER generally was not swollen or otherwise altered. The ground substance of the cytoplasm was not exceptionally electron-dense, although the abundant ribosomes imparted an electron-dense aspect to the cytoplasm of many cells (Fig. 6). Dictyosomes, dictyosome-derived vesicles, coated vesicles, and cytoplasmic vacuoles also were seen in many of these cells (Fig. 6). Small, moderately electron-dense, rhomboid crystals sometimes were present in rough ER (Fig. 8).

Cells that had undergone the most severe degenerative changes usually were found in a region next to the necrotic center of the lesion (Figs. 10–12). Relatively small amounts of intercellular material were seen in this area. Cells generally were moderately to severely collapsed, and cell walls varied from apparently unaltered to swollen. Well-formed cell-wall appositions were observed in some cells (Fig. 11). The characteristic feature of cells in this region was disruption of the tonoplast, which had either disappeared or fragmented to the extent that the cell contents were scattered throughout the cell interior (Fig. 10). The plasmalemma also had become fragmented (Fig. 10) or disintegrated (Fig. 12) in most of these cells.

The nuclear envelope was swollen or ruptured (Fig. 10). Most chloroplasts had lost their bounding membranes and stroma, although the lamellar membranes and their associated plastoglobuli and starch often remained more or less intact (Fig. 10). Chloroplast remnants sometimes also contained relatively large, rounded, electron-opaque fibrous bodies (Fig. 12) quite different from the smaller, rhomboid crystals that were more common in the cytoplasmic remnants (Fig. 12).

Cells in the necrotic centers of the lesions were collapsed and filled with electron-dense remnants of the protoplast (Figs. 13 and 14). Very little electron-dense intercellular material was found in this area of the lesion. Cell walls generally were intact, but localized areas of dissolution sometimes were seen (Fig. 14). Cell-wall appositions were present in some of these cells. Nuclear remains, chloroplast lamellar membranes (sometimes as negative images), starch grains, and the rhomboid crystals usually were the only identifiable structures in the necrotic protoplast. Small to extensive areas that once held crystallized material, presumably removed during specimen preparation, also were observed (Fig. 13).

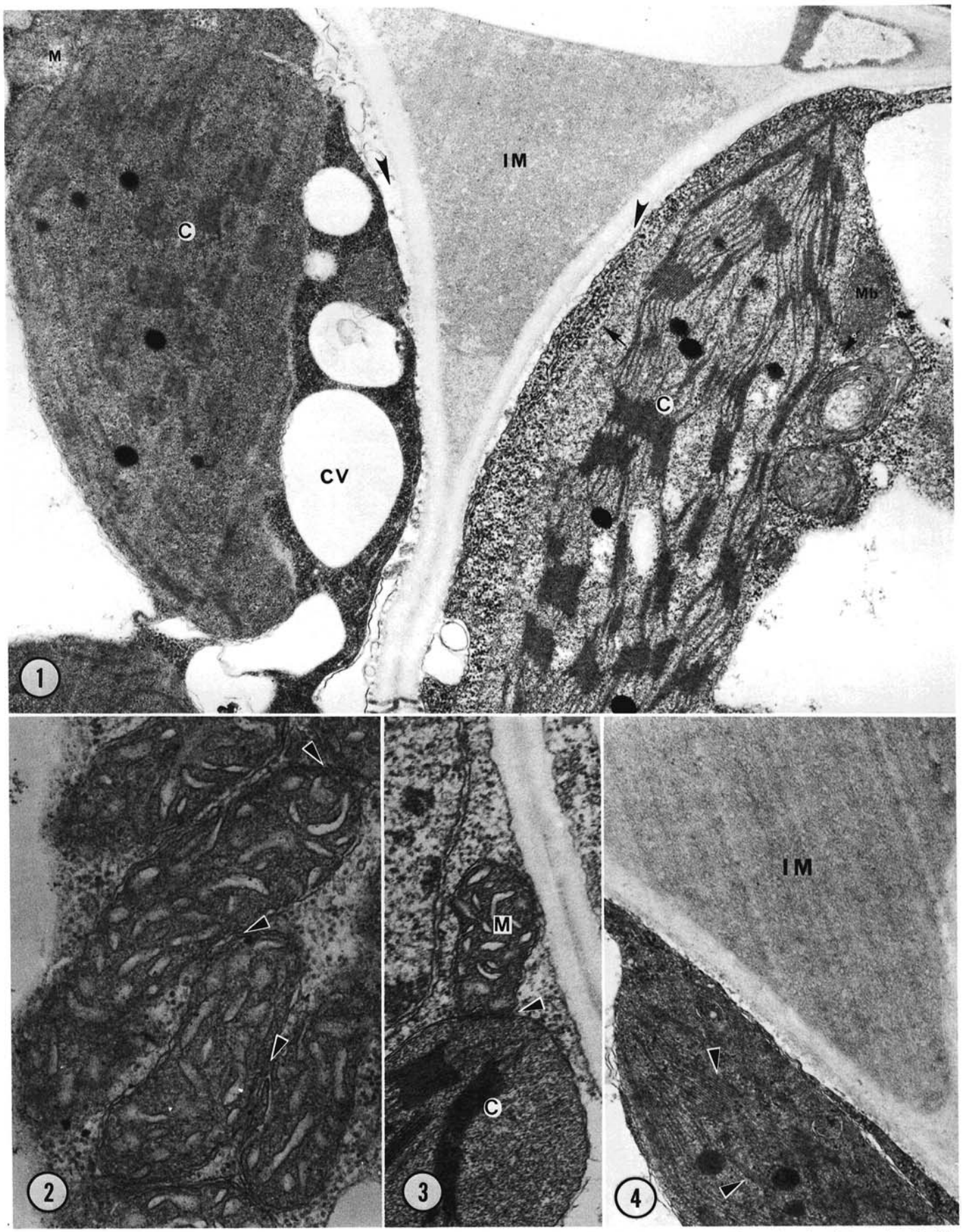
DISCUSSION

Although necrosis occurred in control lesions resulting from application of ethanol and mechanical injury, such lesions differed in several respects from those induced by cercosporin. The necrotic area of control lesions was smaller, and fewer cells around the margin of the necrotic area appeared to have undergone degenerative changes. Intercellular material did not accumulate in the intercellular spaces of control lesions, and no cell-wall appositions were seen. Organelles of degenerating control cells often appeared to lose their ground substance rather than their bounding membranes as was usual in cercosporin-treated cells. Ground substance of the cytoplasm was never observed to become electron-dense in control cells. Other responses, including hyperplastic activity and cell-wall laminations (20) and callose deposition (2,44,60), that have been reported after mechanical or chemical damage to leaf tissue in other species were not seen in control lesions in this study.

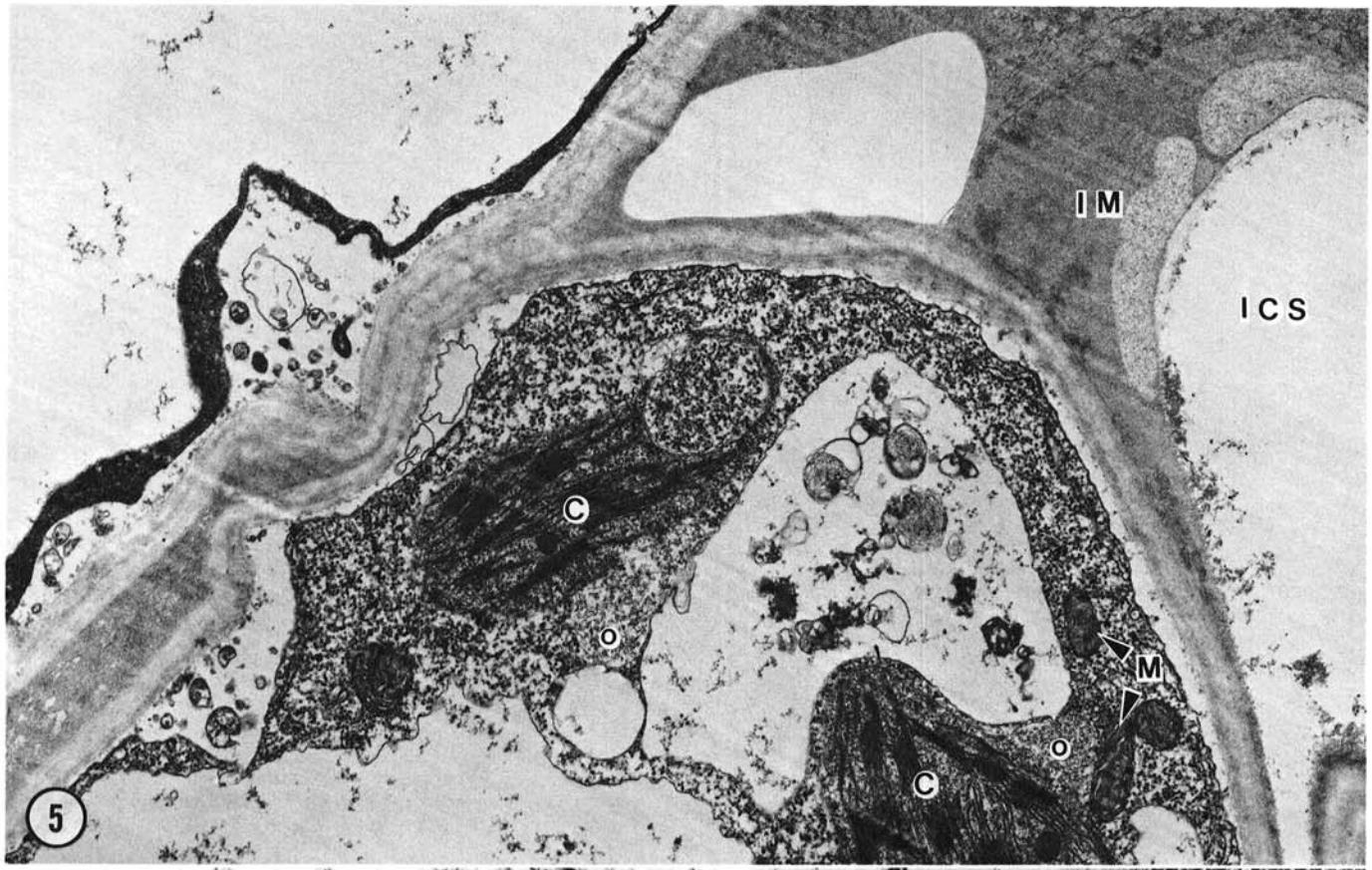
Several cellular effects found in cercosporin-treated tissue have also been described in *C. beticola*-induced lesions (59). One of the most impressive similarities was the occurrence of large quantities of granular, electron-dense intercellular material. In both lesion types, this material was found in wall junctures of adjacent cells or as blebs attached to cell walls. Larger amounts occurred around peripheral or marginal areas of the expanding lesions, often occluding the intercellular spaces.

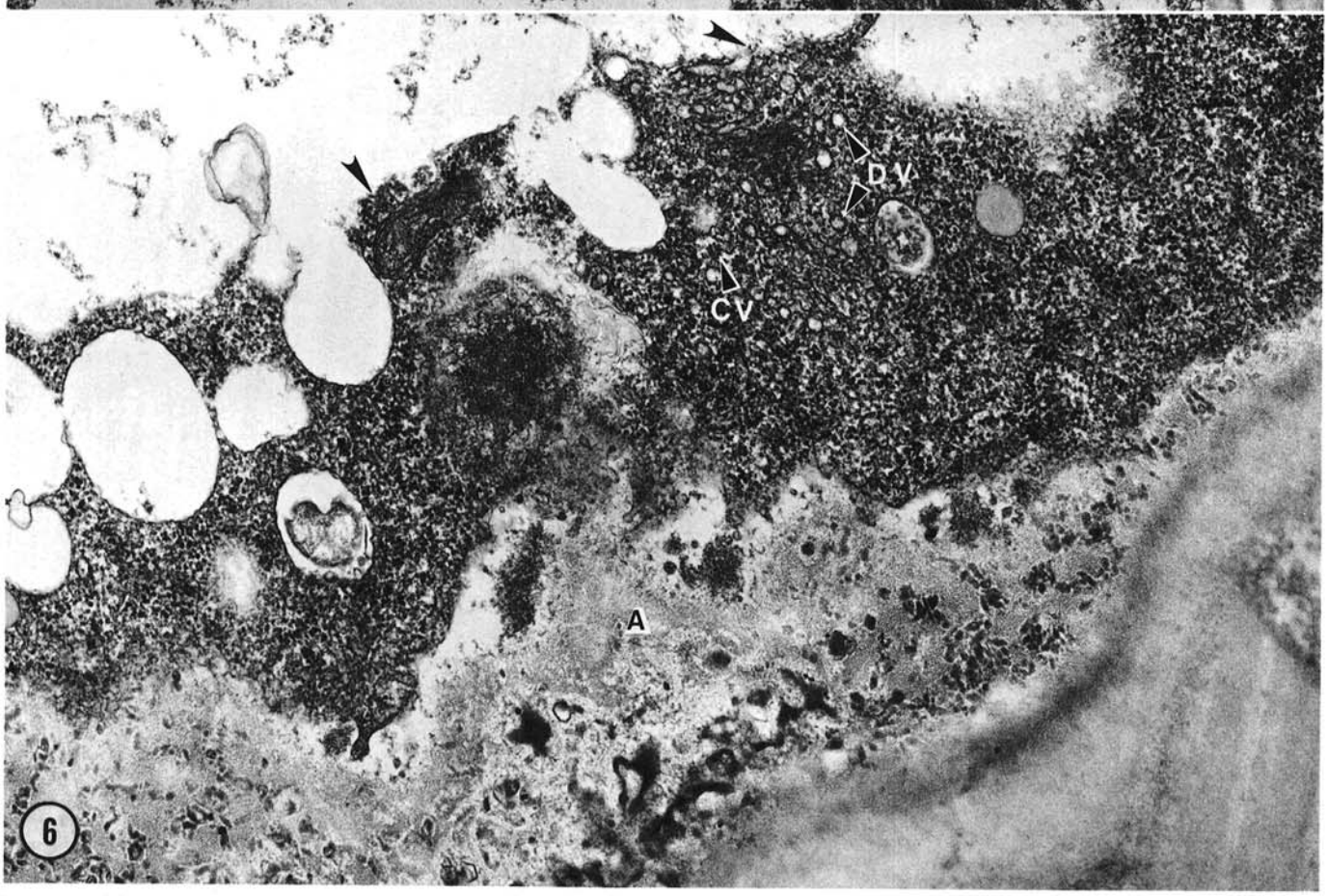
Paramonova (46) described cell-wall protuberances in sugar beet root parenchyma; on the basis of other work, these were believed to be composed of pectic material. Allen (3) reported pectic materials as cell-wall protuberances in the intercellular spaces in marginal areas of orange rust lesions in wheat. This pectic material was thought to be a result of middle lamella breakdown and swelling and gelatinization of pectic materials from the cell wall. Another occurrence of "pectic warts" in crown rust-infected oat was illustrated, but not discussed, by Humphrey and Dufrenoy (27). The intercellular material in *Cercospora*-induced and cercosporin-induced lesions in sugar beet did not appear to be a breakdown product of existing cell walls, however, because cell walls next to or near this material did not appear altered, and often no intercellular material was found near areas where the cell wall obviously had disintegrated.

"Matrix" material superficially resembling the intercellular

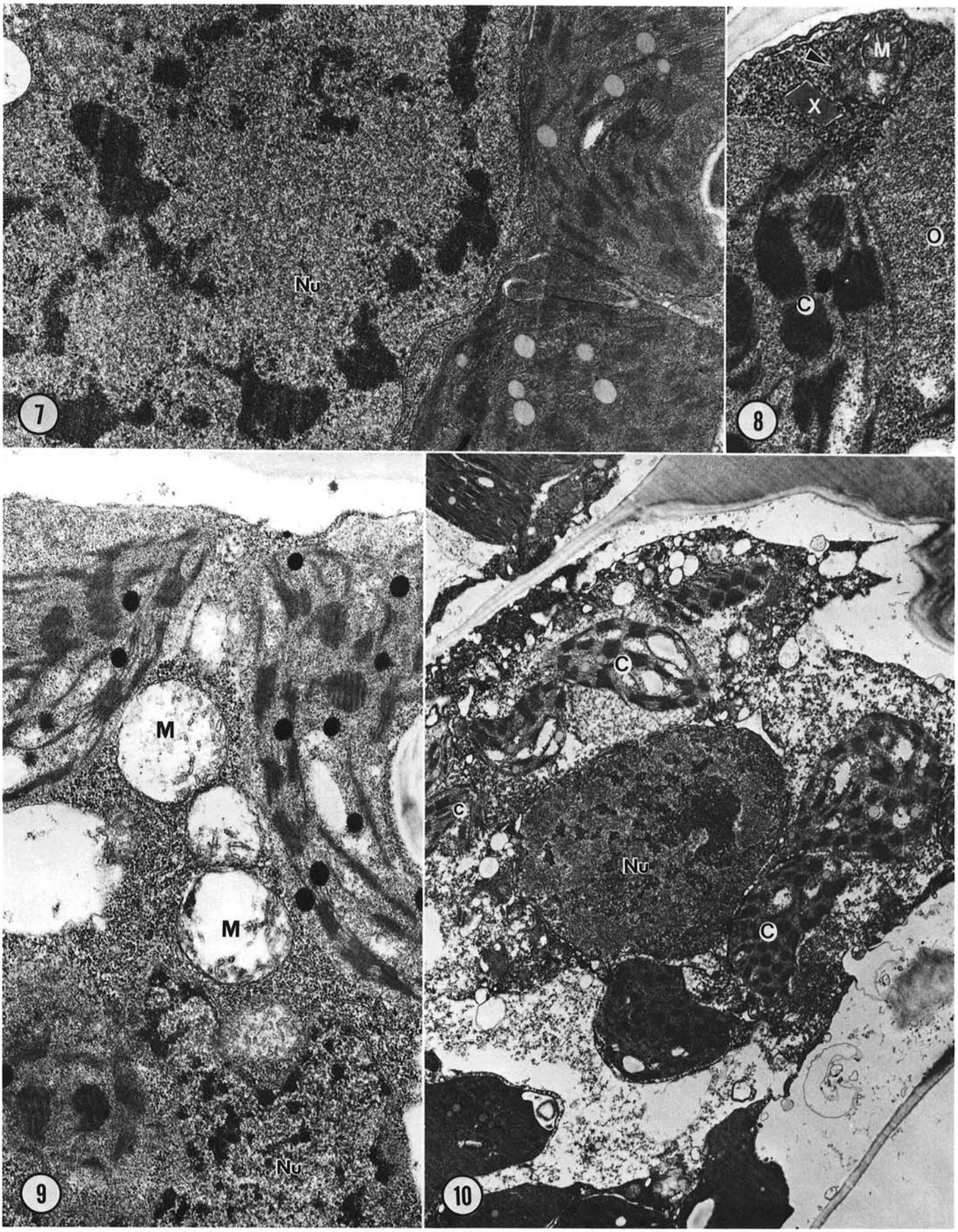


Figs. 1–4. Mesophyll cells from the margins of cercosporin-induced lesions. **1,** The protoplast of each cell has separated from the cell wall (arrowheads). The cell on the left has electron-dense ground cytoplasm, large cytoplasmic vacuoles (CV), and altered chloroplasts (C) and mitochondria (M). The cell on the right has minor alterations, including effects on the microbody (Mb) and chloroplast, and indistinct bounding membranes (arrows). Intercellular material (IM) partly fills the intercellular space (×24,800). **2,** Bounding membranes of a group of mitochondria appear to coalesce (arrowheads) (×49,600). **3,** Chloroplast and mitochondrion bounding membranes adhere to each other or coalesce (arrowhead) (×38,400). **4,** Intercellular material occludes the intercellular space. Chloroplast lamellar membranes (arrowheads) are obscured by the electron-dense chloroplast matrix (×32,000).

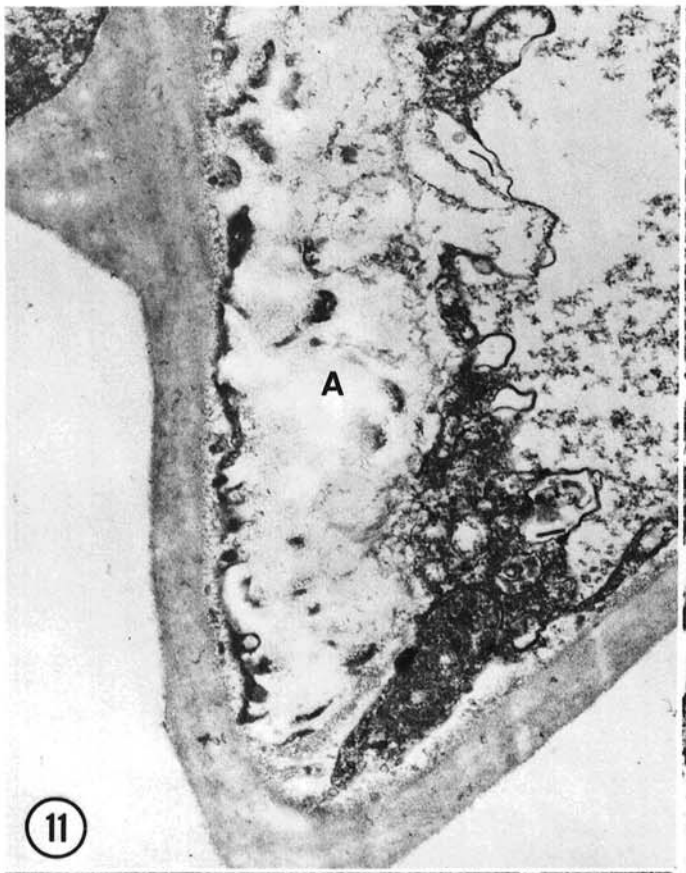


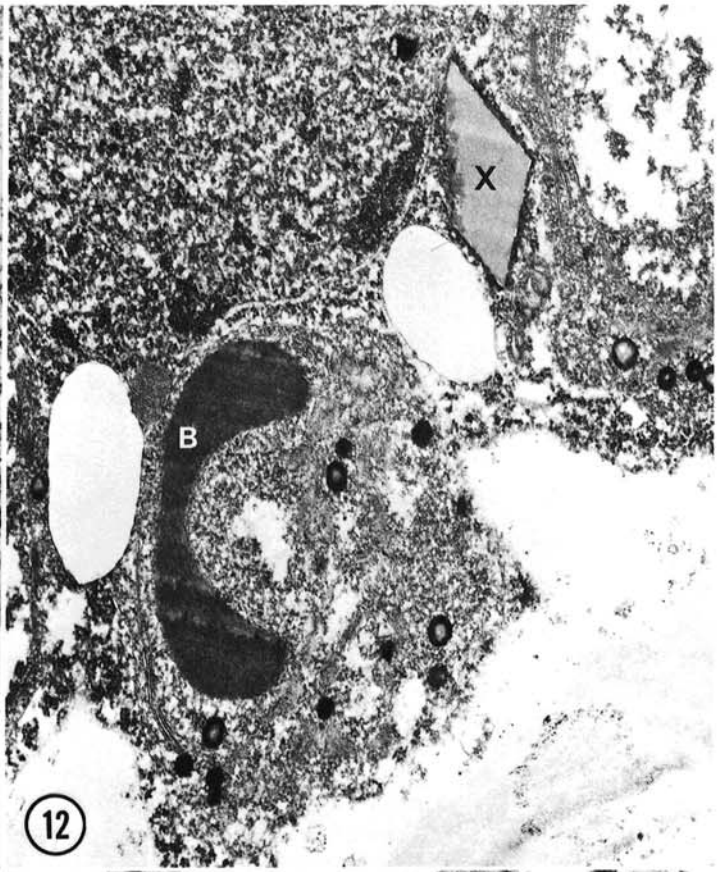


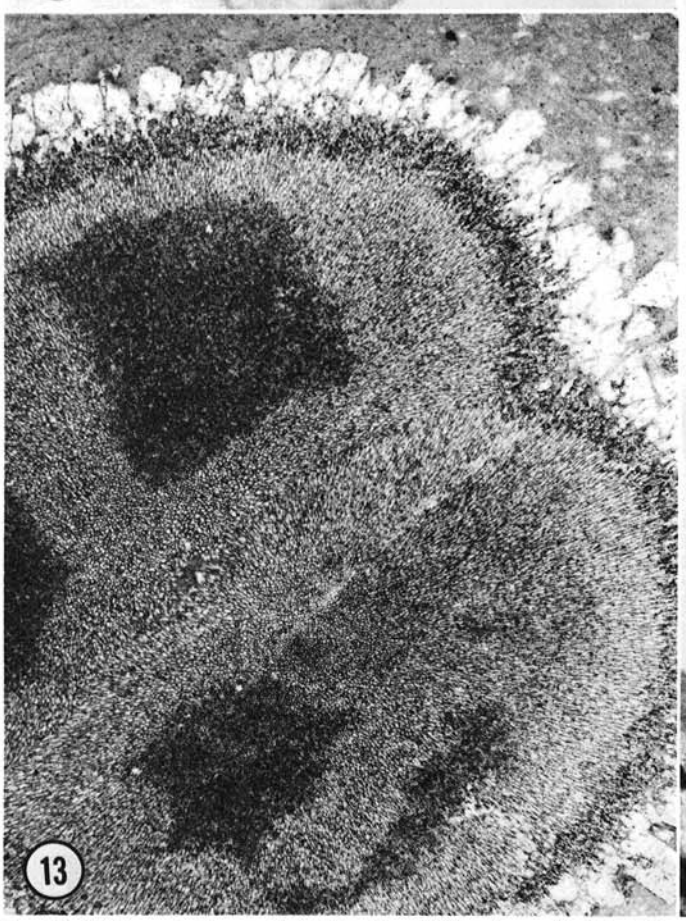
Figs. 5 and 6. Moderately degenerated cells from cercosporin-induced lesions. **5,** Outpocketings of stroma (O) and loss of bounding membranes characterize the chloroplasts (C). Mitochondria (M) have altered internal structure. Intercellular material (IM) is present but does not occlude the intercellular space (ICS) (×19,200). **6,** A large cell-wall apposition (A) is present. The tonoplast is disrupted in localized areas (arrowheads). Dictyosome-derived vesicles (DV), coated vesicles (CV), and ribosomes are abundant (×32,000).

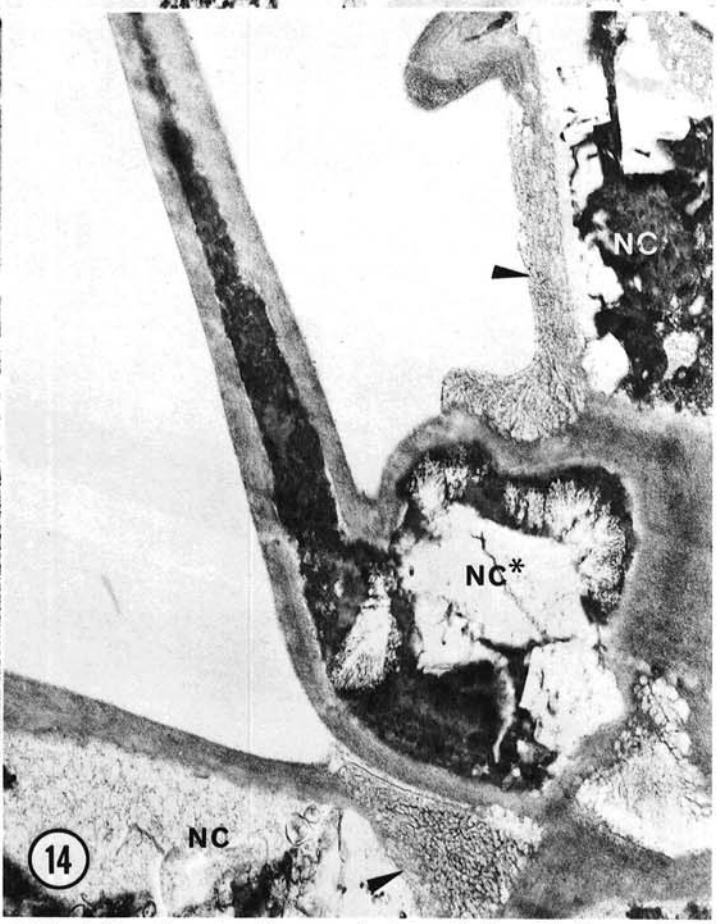


Figs. 7–10. Moderately (Figs. 7–9) and severely (Fig. 10) degenerated cells from cercosporin-induced lesions. **7,** The somewhat irregularly shaped nucleus (Nu) contains condensed chromatin near its periphery (×21,900). **8,** The bounding membrane of a mitochondrion (M) is disrupted (arrowhead), and a rhomboid crystal (X) is present in the cytoplasm. The chloroplast (C) has a large stroma outpocketing (O) (×32,000). **9,** The irregularly shaped nucleus has condensed chromatin scattered throughout its interior. Mitochondria are almost devoid of ground substance (×21,900). **10,** The tonoplast has disintegrated, and cytoplasmic and organellar remnants are scattered throughout the cell interior. Bounding membranes of the nucleus and some chloroplasts are disrupted (×12,400).









Figs. 11–14. Severely degenerated (Figs. 11 and 12) and necrotic (Figs. 13 and 14) cells from the center of cercosporin-induced lesions. **11,** A large cell-wall apposition (A) persists even though the cytoplasm is severely degenerated (×21,900). **12,** An electron-dense fibrous body (B) is present in a disintegrating chloroplast. A rhomboid crystal (X) is present in the cytoplasmic remnants; the plasmalemma and tonoplast have disappeared (×21,900). **13,** The completely necrotic protoplast includes an area that once held crystalline material (×17,500). **14,** Necrotic cells (NC) are collapsed and have areas of localized cell-wall disintegration (arrowheads). The cell marked with an asterisk once held crystallized material (compare with Fig. 13). Note the absence of intercellular material in this area of the lesion (×21,900).

material of this study also has been reported to accumulate in the intercellular spaces of corn leaves resistant to *Helminthosporium maydis* race T (9,11). This material was shown to be proteinaceous (11). Extremely electron-dense ground substance of the cytoplasm was seen in some comparatively healthy cells of each lesion type, but whether this had any significance with respect to the intercellular material was not clear.

A second notable similarity between fungus- and cercosporin-induced lesions was the frequent presence of large, callose-type cell-wall appositions in degenerating cells. The occurrence, structure, and possible functions of such appositions in several other fungus-induced diseases have been reviewed (1,2,10). Appositions also have been observed in other toxin-treated plant tissues (25,33,47–49). *C. beticola* does not penetrate sugar beet cell walls until quite late in the disease process, long after appositions have formed (59). Thus, cercosporin produced in vivo by *C. beticola* is probably at least partly responsible for inducing the appositions seen in fungus-induced lesions.

Effects on cell organelles and membranes were similar in fungal and cercosporin-induced lesions. In less damaged cells, organelle-bounding membranes appeared altered or swollen, and some organelles, especially mitochondria and chloroplasts, appeared to coalesce or adhere to one another. In more severely damaged cells, chloroplast-bounding membranes and eventually ground substance were lost, although the lamellar system and associated starch grains and plastoglobuli were retained. By contrast, the chloroplast lamellar system of *Zinnia* leaves was greatly altered by a semipurified, chlorosis-inducing bacterial toxin (28).

Plastoglobuli sometimes were enlarged in cercosporin-treated tissue but less consistently than in fungus-induced lesions. In moderately and severely toxin- or fungus-damaged cells, the tonoplast had ruptured or disintegrated and organelles or their remnants were scattered throughout the cell interior. This observation contrasts with the condition reported in excised, starved leaf tissue of *Nicotiana*, in which cytoplasts maintained their structural integrity for some time after loss of the tonoplast (51).

Cavallini et al (13) presented a mechanism by which cercosporin could be causing these damaging alterations of membranes. The photodynamic properties of cercosporin (13,34,67) apparently lead to singlet oxygen production, which in turn induces lipid peroxidation and subsequent membrane damage. Cercosporin thus appears to be a photosensitizing dye mediating this process.

Necrotic cytoplasm in both *Cercospora* lesions and cercosporin-induced lesions contained areas that once held crystallized material. This kind of area was not seen in necrotic cells of control lesions but superficially resembles "coagulated areas" reported from a light microscope study of epidermal and parenchyma cells in several plant species infected by *Pyricularia oryzae* (26). Such areas have not been reported in other studies of fungus- or toxin-induced necrotic cells (25,33,47–49).

Rhomboid crystals were found in the cytoplasm of cells from both *Cercospora* lesions and cercosporin-induced lesions. Similar crystals have been found in "washed" beet root tissue disks (23,62) and in excised, starved mesophyll cells of tobacco (51), where their presence in ER has been associated with changes in protein metabolism (23,62) or with cellular degenerative processes (51).

A number of effects attributed to *Cercospora* in the fungus-induced lesions were not found in cercosporin-induced lesions, including electron-dense vacuolar bodies in several stages of the degenerative sequence and in necrotic cells, and electron-lucent "holes" in nuclei, mitochondria, and plastids (59). Although cells ultimately collapsed during the degenerative sequence in cercosporin-treated tissue, this collapse did not appear to be as extensive as in comparable tissue from fungus-induced lesions, and cell walls usually did not form the extensive and tightly curved "loops" characteristic of the necrotic cells of fungal lesions. The generalized cell-wall thickenings in cells of the inner boundary zone of mature 32-day fungal lesions were not seen in cercosporin lesions; however, cercosporin lesions older than 5 days were not examined, and these thickenings occurred late in the development of lesions incited by the fungus.

The large, fibrous bodies found in some degenerating chloroplasts of cercosporin-treated tissue were the only apparent effect of cercosporin treatment not seen in fungus-induced lesions. Superficial similarities exist between these bodies and some others described in the literature, including pseudocrystalline bodies in degenerating chloroplasts of excised, starved tobacco leaves (51) and crystalloid protein bodies found in the stroma of plastids in excised bean leaves (65).

Park (47) suggested that the non-host-specific toxins he studied affected cells differently than the host-specific toxins. Many cell membranes were affected in a general way by the non-host-specific toxins, whereas the host-specific toxins affected primarily the plasmalemma. The host-specific toxin victorin also has been reported to affect the plasmalemma, but other degenerative changes, both within the cell and in the cell wall, have been reported (24,25,33). Cercosporin appears to cause degeneration of many of the cell membranes, particularly the chloroplast-bounding membranes, the plasmalemma, and the tonoplast.

The importance of cercosporin in the interactions of *Cercospora* with its hosts has yet to be fully elucidated. In sugar beet cells, cercosporin certainly appears to induce degenerative changes that parallel some of those induced by *C. beticola*. However, relatively few of the more than 1,200 described species of *Cercospora* (14), and even fewer species of allied genera, have been examined for the ability to produce cercosporin. Some examined species have not produced cercosporin under the test conditions (6,35), and within a species known to produce cercosporin, some strains or isolates may not. However, this assessment is complicated by the great importance of cultural conditions in cercosporin production. Not only is the amount of toxin produced greatly influenced by cultural variables (38,40,41,43; E. G. Ruppel, S. S. Martin, and M. P. Steinkamp, *unpublished*), but different isolates or species are affected differently by the same cultural conditions, and some isolates that produce copious amounts of cercosporin under some conditions apparently produce none under others (E. G. Ruppel, S. S. Martin, and M. P. Steinkamp, *unpublished*). Probably no set of conditions is suitable for definitively determining the ability of several species of *Cercospora* to synthesize cercosporin. Therefore, an apparent inability to produce cercosporin in vitro may signify only that the experimenter has not provided the necessary conditions; whether such conditions exist (either in vitro or in vivo) remains unknown.

As far as is known, cercosporin from any *Cercospora* species appears to be chemically identical to that from any other, and cercosporin obtained from one fungal species can induce lesions on plant species that are not hosts of that fungal species (E. G. Ruppel, S. S. Martin, and M. P. Steinkamp, *unpublished*). Thus, cercosporin may be involved in eliciting some of the disease symptoms; whether it is involved in the initial host-pathogen interactions that determine compatibility or incompatibility is unclear.

Some species of *Cercospora*, including *C. beticola*, produce other secondary metabolites (4–7,12). Some of these compounds might act separately or interact with cercosporin in the production of symptoms in host plants. No cytologic studies of the effects of these materials have been published. However, our initial observations of lesions induced by CBT and the yellow toxin isolated from *C. beticola* by the method of Balis and Payne (8) suggest that these materials produce a sequence of cellular degenerative changes different from the cercosporin-induced changes but similar to some of the fungus-induced changes.

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