Ultrastructure of Potato Stems Infected with Corynebacterium sepedonicum

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ABSTRACT

Stems of potato cultivars Russett and Norgold-Russett were inoculated with a pathogenic isolate of *Corynebacterium sepedonicum*. Ultrastructural investigations showed that organelles and membrane systems of some areas of the wilted petioles were more severely affected than other areas, and bacteria were seen in vascular cells and in intercellular air spaces which had increased in size due to separation of the middle lamella. All membrane systems were significantly altered including the plasma membrane, and excessive amounts of abnormal membranes were present in some cells. Injury was more severe and more extensive in infected Russett petioles than in infected Norgold-Russett petioles. Phytopathology 60:1428-1431.

Corynebacterium sepedonicum (Speick) Dows. produces an acidic phytotoxic glycopeptide with a mol wt of 21,400 (5). The peptide portion makes up about 5% of the toxin molecule and contains nine different amino acids. At least one-half of the glycopeptide is glucose, and several other sugars including mannose and L-fucose also contribute to the structure of the glycopeptide (6). The acidic nature of the toxin is mainly contributed by residues of 2-keto-3-deoxygluconic acid, and it is the carboxyl group of this compound that lends biological activity to the toxin (3). Other physical and chemical properties of the toxic glycopeptide have been reported previously (6).

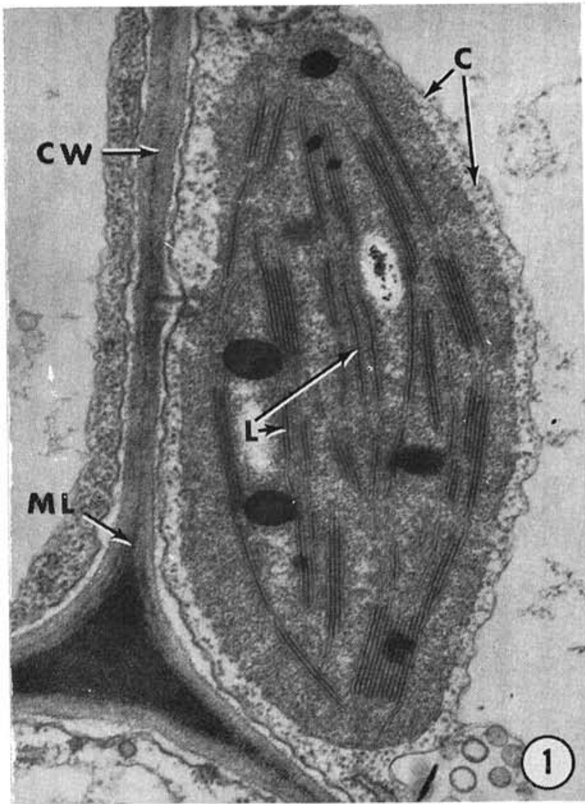
Strobel (6) reported that the phytotoxic glycopeptide of *C. sepedonicum* is a "vivotoxin". He successfully isolated several mg of the toxin from infected Norgold-Russett potato plants and compared the material with the toxin from cultures of the pathogen. The glycopeptides from both sources were identical with respect to their mobility by paper and acrylamide gel electrophoresis, optical rotation, infrared spectra, X-ray diffraction patterns, sugar, organic acid, amino acid content, mol wt as judged by their elution volumes from Sephadex G-200, serological reactivity, and reaction with ninhydrin. Furthermore, the glycopeptide was present in infected plants in quantities large enough to account for symptom production in biological assay tests.

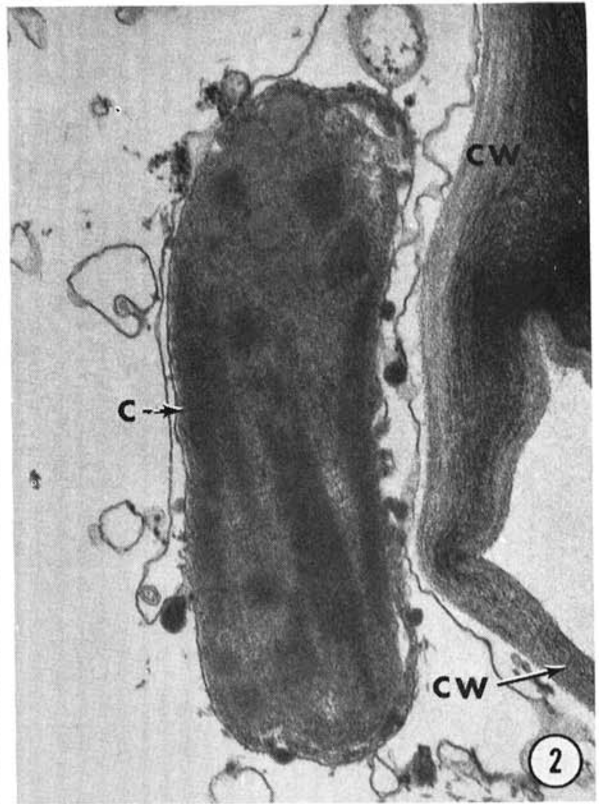
Strobel & Hess (7) presented biochemical, physiological, and ultrastructural evidence that the glycopeptide interferes with the structure and function of the plasma membrane of the plant cell, resulting in wilting of the plant cuttings being tested. Furthermore, Strobel (5) demonstrated that only 40-50 μg of toxin were required to adversely affect plant cuttings. The evidence to date strongly suggests that the toxic glycopeptide plays a role in producing wilting and chlorosis in potato plants showing symptoms of ring rot. The purpose of this report is to lend further credence to this hypothesis by showing that some of the cytological features of potato plants infected with *C. sepedonicum* resemble those produced by the toxin alone.

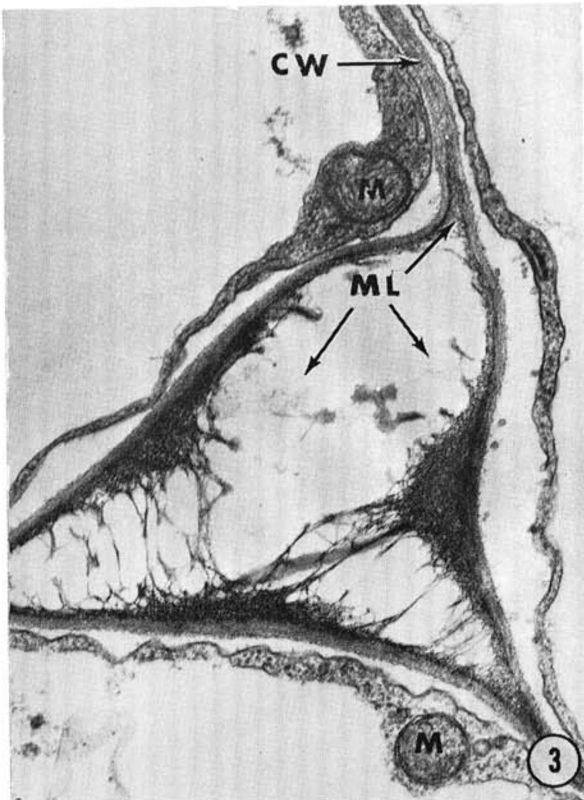
MATERIALS AND METHODS.—In the field, Norgold-Russett tubers are more tolerant to decay than Russett tubers. Seed pieces of these two cultivars were planted in soil contained in 10-inch plastic pots. The plants were grown in continuous lighting under a bank of four 100-watt incandescent bulbs and three fluorescent bulbs with the temp fluctuating between 23-27 C. Several days after the shoots emerged from the soil, they were inoculated with a pathogenic isolate of *Corynebacterium sepedonicum*. Inoculation was performed by placing a loopful of cells, obtained from a culture of the organism growing on an agar slant (6), into a 0.5-cm incision made at the base of the plant stem. Samples of leaf petiole tissue showing symptoms of wilt and chlorosis were taken 4 to 5 weeks after inoculation. The petioles were removed with a sharp razor blade, cut into 1- to 2-mm sections, and placed in 3% glutaraldehyde and 3% acrolein buffered with 0.2 M sodium cacodylate buffer (pH 7.2-7.4). Other sections were surface treated in 70% ethanol, flamed, and plated onto petri plates containing agar medium (6) to verify the presence of the pathogen. Cultures were maintained at room temp, and the gram stains were made after 5-7 days of incubation. Petiole sections were fixed and embedded according to the glutaraldehyde-acrolein-osmium procedures of Hess (1), modified only by a longer fixation time due to the necessity of transporting fixed material by mail. Noninoculated plant material served as controls.

RESULTS.—*Norgold-Russett.*—Noninfected plants contained characteristic mitochondria with typical cristae. The membrane systems of the cell were generally distinct and continuous. Lamellae were easily distinguished in chloroplasts (Fig. 1), and the middle lamella was more electron-dense than other layers of the cell wall. The electron density of the middle lamella was particularly evident at the union of three cells (Fig. 1).

Some organelles of infected cells were not visibly altered, while others were affected adversely. Some areas of the stems were more severely affected, but these areas could not be positively correlated with petiole morphology. The effects of bacterial infection







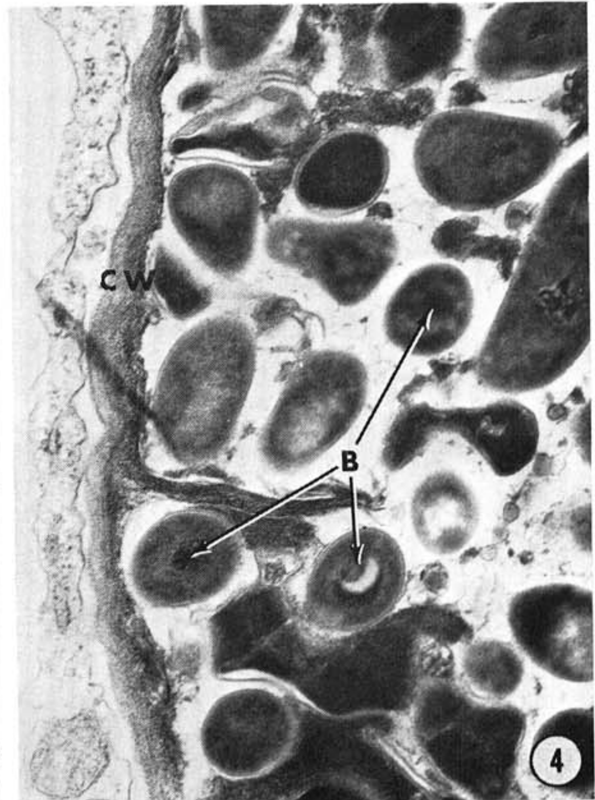
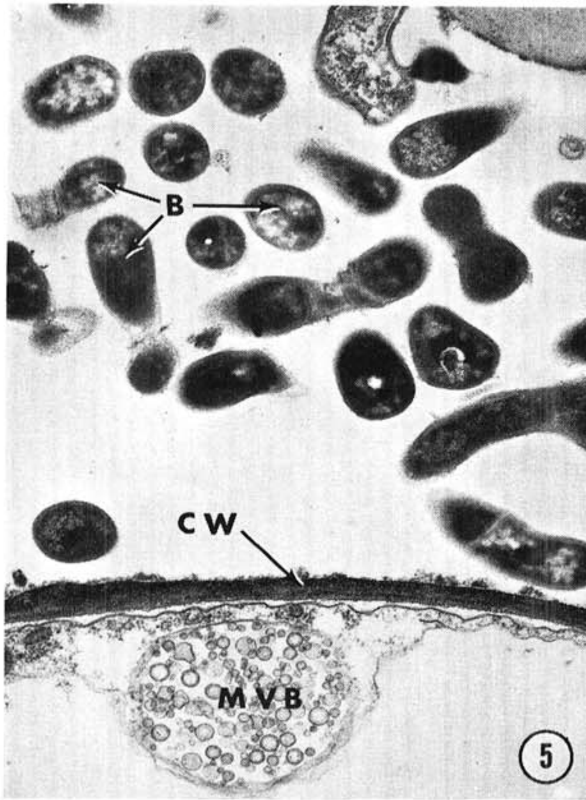
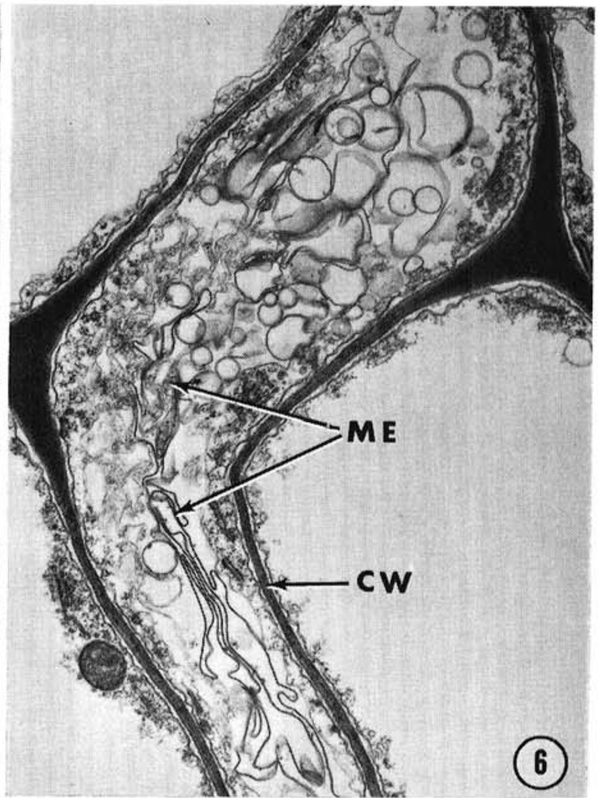
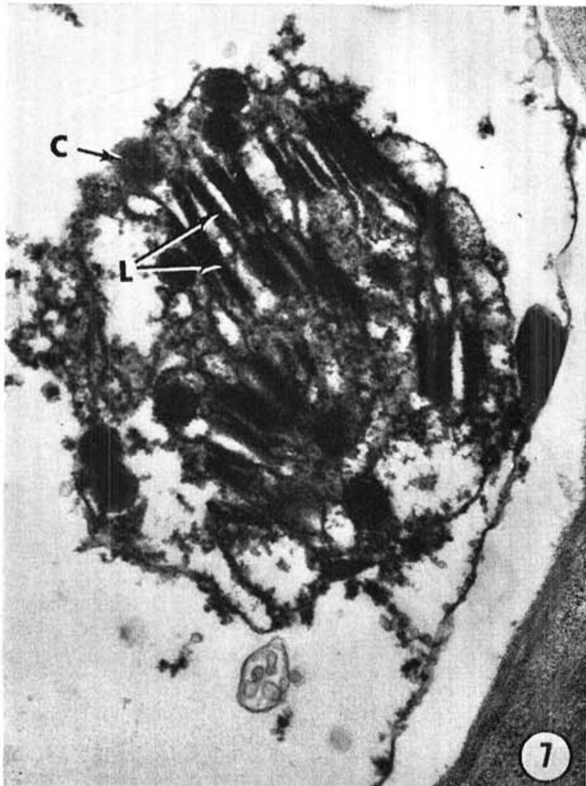


Fig. 1-4. Norgold-Russett potato petioles. **1)** Portions of petiole cells from a control showing a chloroplast (C) with lamellae (L), the cell wall (CW), and middle lamella (ML). (×25,000) **2)** Portions of petiole cells from infected petiole showing the cell wall (CW) and an altered chloroplast (C). (×29,000) **3)** Portions of petiole cells from infected stem showing a middle lamella (ML) which has broken open, mitochondria (M) with indistinct cristae, and the cell wall (CW). (×25,000) **4)** Portion of a petiole cell from infected petiole showing the cell wall (CW) and bacteria (B) in the middle lamella. (×22,000)







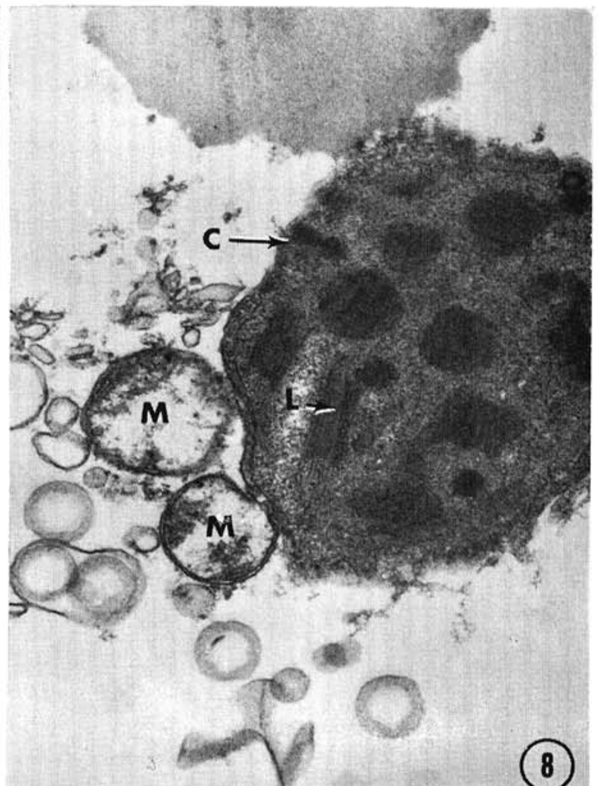


Fig. 5-8. Infected Norgold-Russett potato petiole. **5)** Portions of petiole cells showing a cell wall (CW), bacteria (B) in a vascular cell, and a multi-vesicular body (MVB). (×17,600) **6)** Portions of petiole cells showing a cell wall (CW) and excessive amounts of abnormal membranes (ME) in a stem cell. (×9,000) **7, 8)** Infected Russett potato petioles. **7)** Portion of a petiole cell showing an altered chloroplast (C) with separating lamellae (L). (×26,100) **8)** Portion of a petiole cell showing an altered chloroplast (C) with intact grana but indistinct lamellae (L), and mitochondria (M) with indistinct cristae. (×23,900)

on chloroplasts (Fig. 2) and mitochondria (Fig. 3) were particularly evident in some areas of infected stems. Altered chloroplasts characteristically lacked recognizable lamellae, and the double unit membrane which commonly surrounds them was lacking or indistinct. Although affected mitochondria retained size and shape of typical mitochondria, they generally lacked the characteristic cristae which are present in mitochondria in noninfected stems.

The separation of the middle lamella at the union of three cells (Fig. 3) is a distinctive feature of infected stems. These areas were commonly continuous with the intercellular air spaces. Bacterial cells were sometimes seen within the separated middle lamella (Fig. 4). Bacteria were also present in vascular conducting cells (Fig. 5). In some stems, organelles were only mildly affected in cells adjacent to cells containing bacteria. In other infected petioles, organelles were adversely affected in a large proportion of the petiole cells. Bacteria isolated from comparable sections of plant material were gram-positive, and resembled the morphological features of *C. sepedonicum* originally used to inoculate the plants. Multi-vesicular bodies (Fig. 5) were commonly larger and more numerous in infected petioles, and in severely affected areas of the petiole, cell walls lost their distinctive pattern of layering.

Membrane fragments were characteristically observed, and membranes were not continuous in affected parts of infected petioles. Disrupted membranes were a common characteristic of infected petioles (Fig. 2). Some cells contained excessive amounts of abnormal membranes (Fig. 6). All membrane systems were significantly altered, including the plasma membrane.

Russett.—Noninfected control plants contained characteristic, normal-appearing organelles which closely resembled the control plants of Norgold-Russett. Infected Russett plants were affected in a manner similar to Norgold-Russett plants, except that the injury was more severe and more extensive in Russett plants. Some chloroplasts appeared to disintegrate (Fig. 7), and separation of lamellae was evident, while other chloroplasts appeared to remain intact (Fig. 8), but the lamellae were not distinct, and the surrounding membranes were not evident.

DISCUSSION.—This study indicates that all of the membrane systems of the cell were affected by *Corynebacterium sepedonicum* infection. These findings are in agreement with the observations of Strobel & Hess (7) that a toxic glycopeptide from this bacterium destroys the integrity of cellular membranes, resulting in a net loss of water. Organelle breakdown and membrane disruption were not confined to a given area, indicating that widespread damage can be caused in spite of cellular morphology.

Organelle disruption apparently does not correlate positively with presence of bacteria, since organelles in cells next to cells containing bacteria are often not altered appreciably, while organelles several cells from visible bacteria are commonly affected. This suggests that the toxin or other products or both are readily diffusible and are able to alter host organelles several cells from the presence of the bacterial cells. Previous investigations showed that organelles of host cells were significantly altered when toxic glycopeptide was used alone (7). The present investigation has shown that naturally wilted plants have similarly altered organelles. These findings are compatible with the findings of other investigators who have worked with parasitic fungi and plant-host relationships. Reichle (4) showed that cell walls of bean hypocotyls were degraded in advance of *Fusarium solani* f. *phaseoli* hyphal cells, and that the host protoplast breaks down before it comes in contact with the parasite. Hess (2) showed that cell disruption occurred several cells from the pink root fungus (*Pyrenochaeta terrestris*) during infection of onion roots, and that membrane disruption was a common feature of the infection relationship.

Hess (2) also showed that abnormal membrane accumulation was commonly evident with the onion root rot-fungus association he studied. Excessive amounts of abnormal membranes were also present in infected potato stems, possibly reflecting a degenerative change in host cells.

Sections for ultrastructural investigation were cut large enough to examine all cells from the outside to the center of stems. In some instances, vascular cells contained large numbers of bacterial cells but vascular plugging was not seen. Bacteria were also observed in the middle lamella, which commonly becomes continuous with the intercellular air spaces in infected cells. The mechanism of entry into the middle lamella is not known, but structural integrity of cell-wall layers must have been destroyed before entry could take place, indicating that cell walls are significantly altered. In severely affected areas of petioles, cell-wall layering was visibly altered, which is in agreement with previous findings (7).

The cells and organelles of all infected Russett stems examined were more severely affected than infected Norgold-Russett petioles. Although inherited biochemical differences are expected from one variety to another, specific reasons for these differences are not known.

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