Histochemical Changes in Oat Cell Walls After Victorin Treatment

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ABSTRACT

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Oat roots treated with victorin showed greater affinity for staining with Schiff's reagent, silver methenamine, and an alkaline bismuth reagent than did comparable nontreated roots, but no differences were found by using Thiery's technique. Staining of cell walls, the outer surface of the plasmalemma, starch grains, and Golgi vesicles was reduced by aldehyde-blocking reagents in victorin-treated roots, suggesting that changes in carbohydrates were responsible for the differences observed. Cell wall lesions contained amorphous materials similar in density to the cell wall with all stains used. These results strengthen the hypothesis that wall lesions may result from activation of host cell wall enzymes.

Additional key words: toxin, ultrastructure.

The mode of action of victorin, the phytotoxic, host-selective product of *Helminthosporium victoriae* Meehan and Murphy, is unknown. Rapid losses of electrolytes after exposure of susceptible oats to this toxin suggest permeability changes as one of the first effects. However, there are other effects which suggest this toxin must enter cells to be lethal (14). Ultrastructure studies have shown that drastic changes in cell walls and increases in Golgi activity precede detectable changes in membrane structure in victorin treated roots. Cell wall lesions contain amorphous materials that appear to originate within the wall, although vesicles contribute to their enlargement (5). The first detectable ultrastructural effect is an increased electron density of cell walls fixed in potassium permanganate. This change was not observed after fixation in osmic acid. The former fixative is a stong oxidizing agent and is reduced mainly to manganese dioxide by monoamines, hydroxyl groups, or molecules with double bonds (6).

To further define the nature of effects of victorin on cell walls and cellular organelles, several histochemical methods, some adapted for electron microscopy, were employed. Some of the results reported here have been discussed briefly in reviews (4,15).

MATERIALS AND METHODS

For light microscopic studies, oat seedlings of three susceptible cultivars, Victorgrain 48-93, Fulgrain, and Park were exposed to water or 1 unit/ml (9) of victorin for 14 hr. Two resistant cultivars, BRM-282 and Red rust-proof were exposed to water or 50 units/ml of victorin for 24 hr. Histochemical tests for cellulose, pectins, and lignins were made on fresh tissues by procedures described by Jensen (7).

For ultrastructure studies, seedlings (cultivar Park) were exposed to victorin (1 unit/ml) or water for 3 hr or to victorin (0.1 unit/ml) or water for 24 hr. Roots were then rinsed in water and the apical 2 mm fixed in 4% purified glutaraldehyde in 0.1 M potassium phosphate buffer at pH 7.1. Post-fixation in osmic acid was omitted because osmium interferes with localization reactions when periodic acid oxidation is not used (6). After dehydration in ethanol, the segments were embedded in Epon. Thin sections were mounted on gold grids and subjected to the following treatments:

Silver methenamine technique. Silver methenamine was prepared by the method of Swift (13). Grids were floated on a 1% solution of periodic acid for 30 min at 21 C and rinsed for 3 min in distilled water. They were then submerged in silver methenamine and placed in darkness for 1 hr in a 60 C oven unless otherwise stated. After staining, the sections were rinsed with 2% sodium thiosulfate for 15 min to remove ionically-bound silver. Modifications of this technique were as follows:

(A) Periodic acid oxidation was omitted.
(B) Grids were treated with dimedone, phenylhydrazine or semicarbazide to block aldehydes. These reagents were prepared as previously described (2,11). However, in repeated tests the formerly recommended temperature for these blockages (60 C) was judged to be unsatisfactory since solvent alone reduced silver deposition. Thus, blockage was conducted for 2 days at room temperature.
(C) Grids were treated with iodoacetic acid for 2 days at room temperature to block sulfhydril groups (11).

Thiery technique. The method of Courtoy and Simar (3) was used. Modifications of this technique were:

(A) No periodic acid oxidation.
(B) No thiocarbohydrazide treatment.
(C) No oxidation or thiocarbohydrazide.

Alkaline bismuth technique. The reagent was prepared according to Ainsworth et al (1). After oxidation for 15 min with 3% periodic acid, the grids were stained 1 hr at 36 C and washed.

RESULTS

Light microscopy. No differences were found between susceptible or resistant roots placed in water or victorin solutions and stained with IKI-H_2SO_4, ruthenium red, methylene blue, hydroxylamine-ferric chloride, or phloroglucinol. Nonoxidized roots of susceptible oats treated with victorin were strongly reactive with Schiff's reagent.

Silver methenamine. Sections from roots treated with victorin for 3 hr showed a strong reaction with silver methenamine without prior periodate oxidation (Fig. 1). Dense granules were deposited on chromatin, ribosomes, and in the cell walls, but organelle membranes were completely unstained. The most dense depositions were found in epidermal, outer cortical, and root cap cells. Roots treated with the toxin for 24 hr stained densely throughout all regions but the intracellular localization was the same as that of the shorter treatment. Wall lesions, which were more common in the 24 hr treatment, also accumulated silver stain (Fig. 3). However, in contrast to uniform staining of cell walls, lesions contained much unstained material.

Compared with victorin-treated roots, sections from control roots showed little silver deposition without prior oxidation (Fig. 2). Intracellular deposition was similar to that of toxin-treated roots, but in most cases staining was restricted to faint deposits along the cell wall. The depositions shown in Fig. 2 represent the most intense reaction seen in nonoxidized control roots.

In sections blocked with dimedone, ribosome and chromosome

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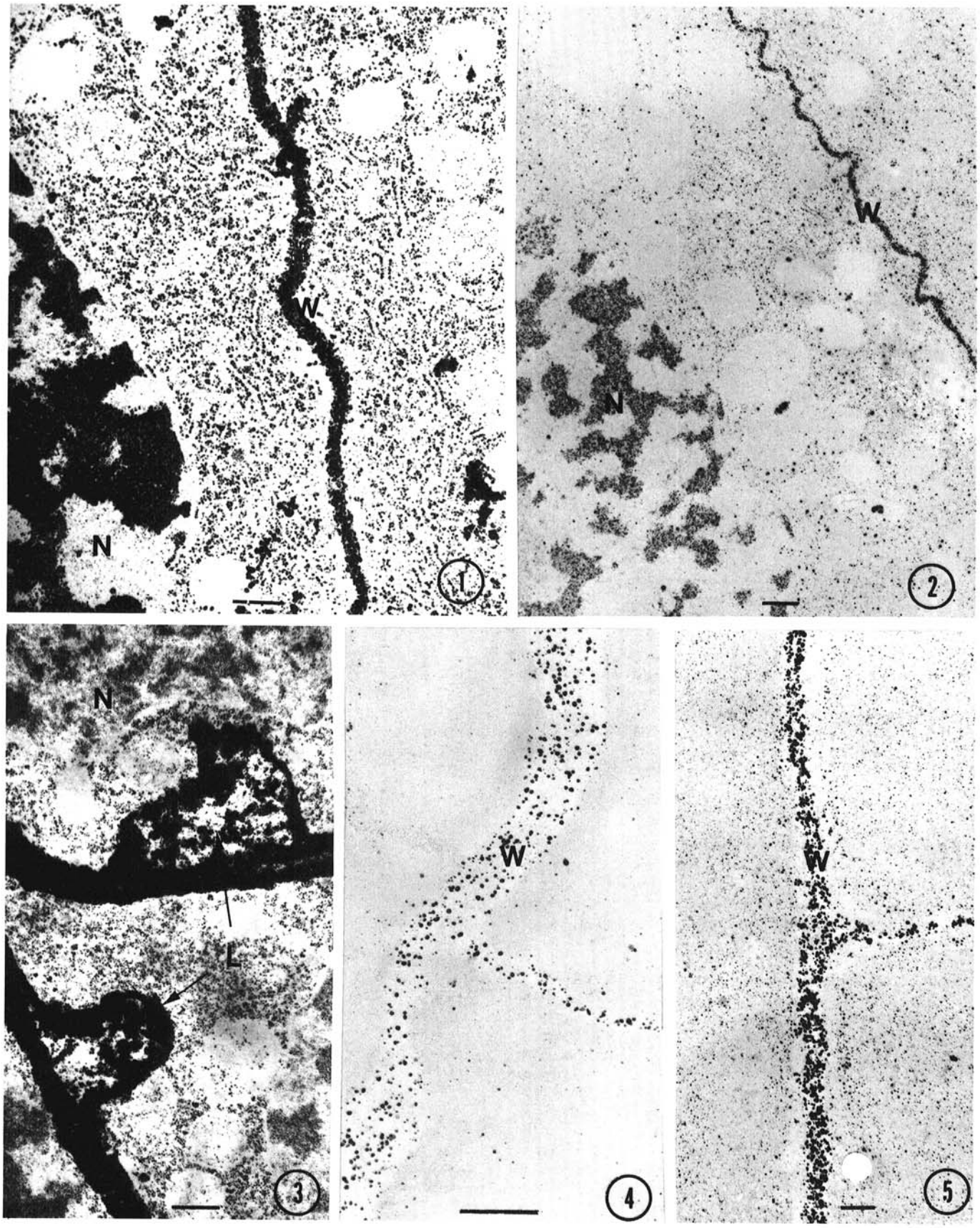


Fig. 1-5. Cortex cells in nonoxidized sections of oat roots stained with silver methenamine. **1,** Root treated with victorin 3 hr shows heavy silver depositions in the nucleus, cell wall, and ribosomes. **2,** Faint stain deposition in cells of control root. **3,** Cell wall lesions in roots after 24 hr in victorin show silver deposition. **4,** Dimedone treatment of section from victorin-treated root decreased wall staining. **5,** Phenylhydrazine blockage of section from victorin-treated root decreased but did not eliminate silver deposition in walls. L = wall lesion, N = nucleus, W = cell wall. Scale bar represents 0.5 μm.

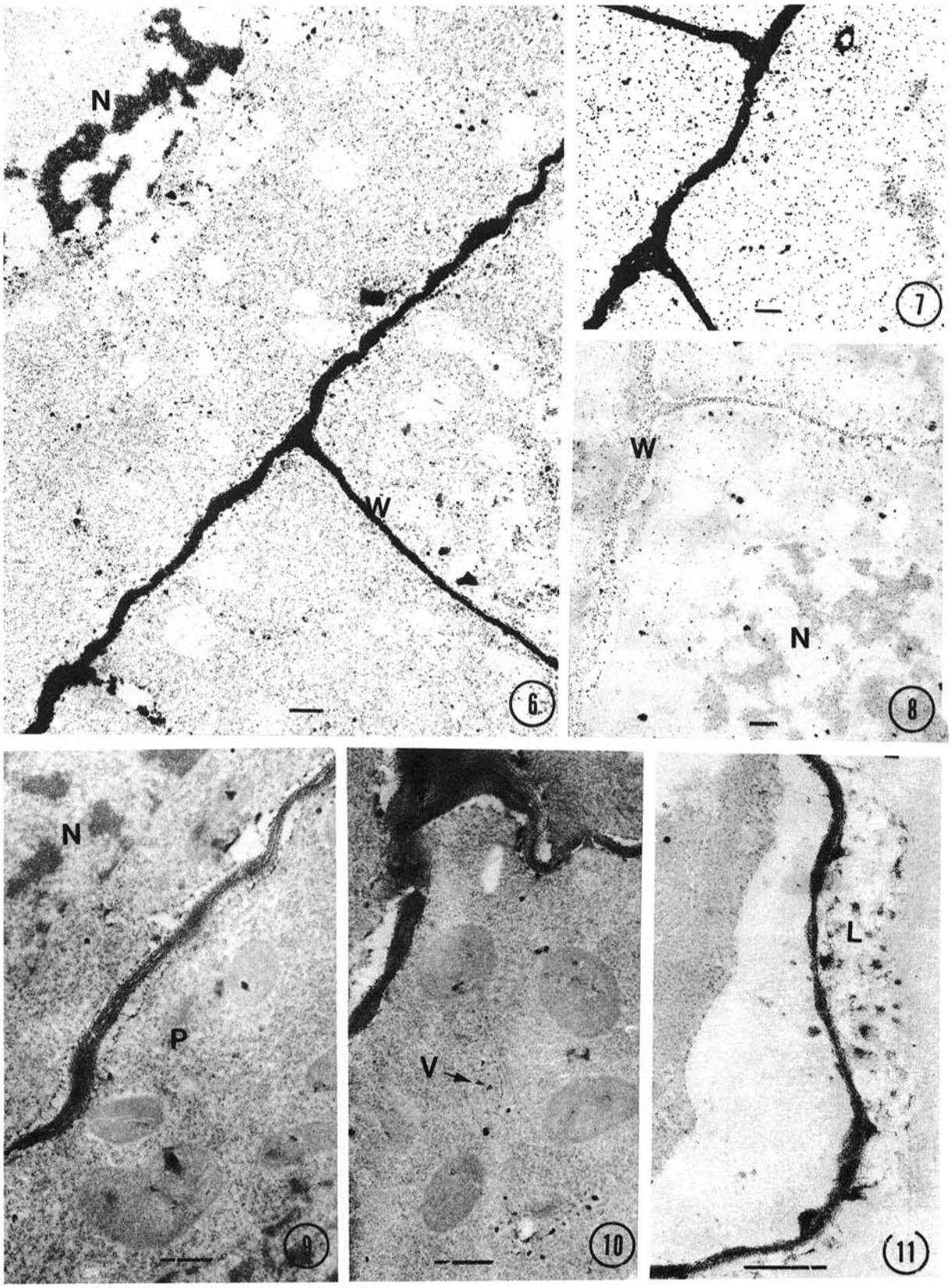
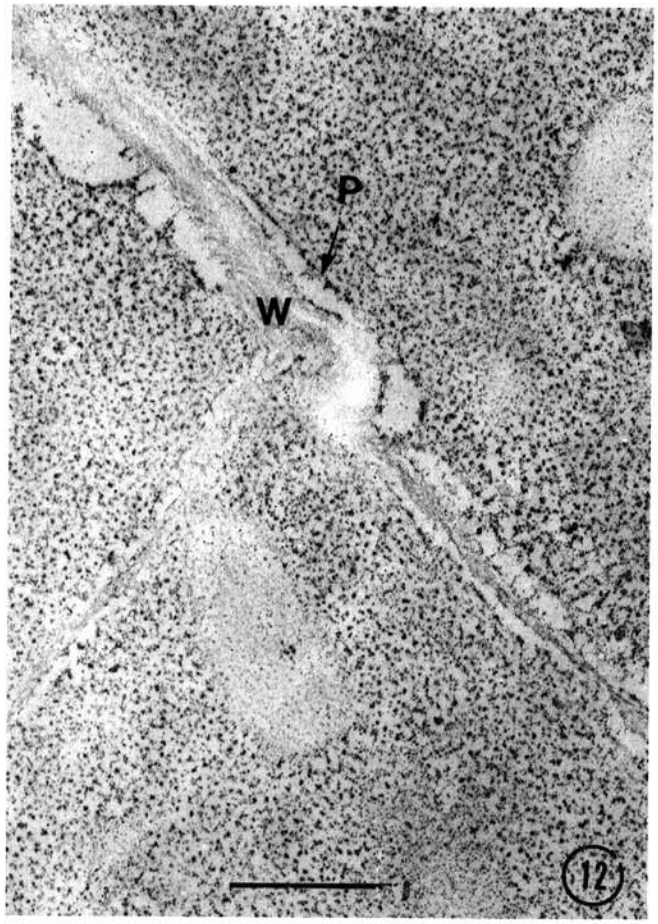
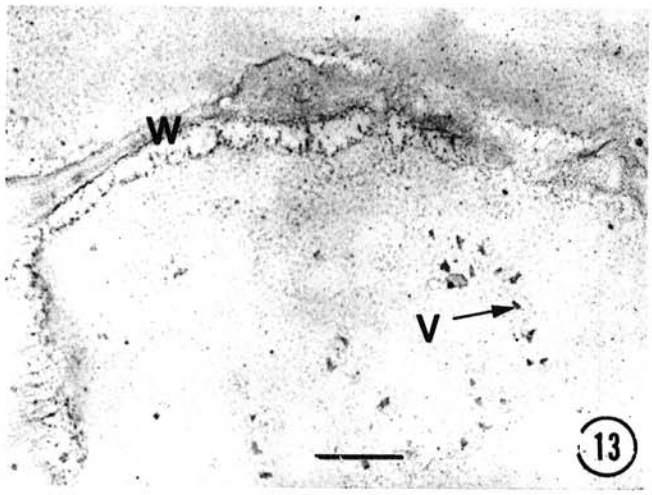
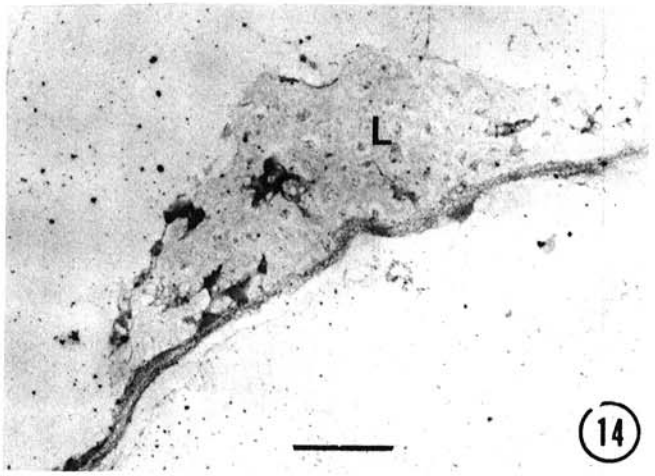


Fig. 6-11. The effect of periodic acid oxidation on silver methenamine. **6,** Cells from control roots show enhanced deposition on the nucleus, ribosomes, and cell wall. **7,** Oxidized section from victorin-treated root stained with silver methenamine 30 min showed decreased nuclear staining and increased wall staining. **8,** Oxidized section from victorin-treated root blocked with dimedone prior to staining 30 min with silver methenamine. Fig. 9-11. Staining reactions of sections stained by the Thiery procedure. **9,** Electron-dense deposits in control roots are associated with the plasmalemma and fibrillar materials between the wall and membrane. **10,** Golgi vesicles in the third cortex layer are reactive in victorin-treated root. **11,** Epidermal wall lesion contains both reactive and nonreactive material in root treated with victorin. L = wall lesion, N = nucleus, P = plasmalemma, V = vesicle, W = cell wall. Scale bar represents 0.5 μm.







staining was eliminated and the reaction in cell walls was decreased (Fig. 4). Semicarbazide completely prevented the stain reaction. Both of these reagents decreased staining to the extent that toxin-treated and control roots were indistinguishable. Phenylhydrazine also reduced silver staining (Fig. 5), but was not as effective as dimedone or semicarbazide. These results suggest that victorin-treated roots have a high aldehyde content. Iodoacetic acid treatment had no effect on silver deposition in either controls or victorin-treated roots.

Periodic acid oxidation of sections from control roots resulted in heavy silver deposition (Fig. 6) which appeared very similar to that of nonoxidized victorin-treated roots (Fig. 1). Periodic acid oxidation of sections from victorin-treated roots resulted in decreased chromatin and ribosome staining. However, cell wall staining was so greatly intensified that staining time was decreased to 30 min (Fig. 7). Dimedone blockage of oxidized control or toxin-treated sections decreased silver deposition in walls equally (Fig. 8).

Thiery reaction. Silver deposition did not occur in nonoxidized sections treated with thiocarbohydrazide and silver proteinate or in oxidized sections treated with silver proteinate alone. Sections from both toxin-treated and control roots had a similar degree of silver deposition when treated by the complete Thiery procedure (Fig. 9,10). The plasmalemma was sharply delineated by the staining, and staining of fibrillar or granular materials was located between the membrane and the darkly-stained cell wall. The granular deposits in cell walls were finer than those observed when the silver methenamine technique was used. Golgi vesicles were reactive in all regions of toxin-treated roots (Fig. 10). Densely stained Golgi vesicles were confined to the epidermal and outer root cap cells of control roots. Differences in Golgi activity previously were detected in roots fixed in potassium permanganate (5) and were more readily apparent when the Thiery technique rather than the silver methenamine technique was used. Mitochondria and plastids also were more distinguishable with the Thiery technique than with silver methenamine and frequently contained several dense deposits. Wall lesions contained densely-stained material (Fig. 11) but were not as heavily stained as with silver methenamine (Fig. 3).

Alkaline bismuth technique. Nonoxidized sections from toxin-treated or control roots did not stain. Extremely fine deposits occurred on cell walls, on the plasmalemma, and upon ribosomes in oxidized sections. In control sections, wall staining was most dense in epidermal and root cap cells (Fig. 12) whereas interior walls of toxin-treated roots also were stained (Fig. 13). The differences were not as pronounced as those with the silver methenamine stain. In healthy roots, staining of Golgi vesicles was confined to epidermal and root cap cells, but also occurred in interior cortical cells of victorin-treated roots (Fig. 13). Other organelles were completely unstained and were difficult to resolve. Fibrillar materials between the wall and membrane resemble those found with the Thiery technique. Cell wall lesions contained amorphous, densely-stained materials (Fig. 14).

DISCUSSION

Results indicate that victorin treatment either increases the exposure of oxidized sites or increases their numbers. Many of these are associated with cell walls; however, detection of this change is dependent upon the cytochemical technique used. Silver methenamine was used by Pickett-Heaps to localize carbohydrates in plant tissues (11). Metallic silver is deposited from a reduction

Fig. 12-14. Effects of victorin on the staining reactions of oat cell wall (W) sections exposed to alkaline bismuth. **12,** The first cortical cell in an untreated control root shows a faint wall reaction and denser deposits on the plasmalemma (P), fibrillar materials between the wall and membrane, and on ribosomes. **13,** Staining of a section from victorin-treated root is essentially similar except that the staining of Golgi vesicles (V) is evident. **14,** Epidermal wall lesion (L) in victorin-treated root contains materials of differing stain affinity.

reaction involving aldehyde groups produced by periodic acid oxidation of α-1,2-glycols or α-amino alcohols. In the present work, nonoxidized sections from victorin-treated roots were highly reactive with silver methenamine. The results with aldehyde blocking reagents indicated that reactivity resided primarily in aldehyde groups.

Silver methenamine staining of periodic acid oxidized control roots was comparable to nonoxidized toxin-treated roots. The staining of chromatin and ribosomes, however, indicates that this method cannot be used solely for the localization of carbohydrates in tissues. Known carbohydrate components, including cell walls, starch grains, Golgi vesicles, and the outer surface of the plasmalemma, were reactive. These same structures stained in victorin-treated roots whether or not oxidation was employed, but oxidation increased the staining intensity. It is unlikely that glutaraldehyde fixation is responsible for the increased staining in victorin-treated roots since corresponding differences were found by using Schiff's reagent on nonfixed tissue.

Differences between healthy and diseased tissues were not as pronounced when the alkaline bismuth technique was used to detect periodate-reactive sites. The primary difference between controls and victorin-treated roots was the staining of interior cell walls in the latter. Previously it was shown that victorin treatment of roots causes several changes in interior cells which make them resemble exterior cells, particularly a change in Golgi secretory activity (5). The results with alkaline bismuth strengthen the hypothesis that oxidizable sites are present in greater numbers or are more accessible to staining after toxin treatment.

Alkaline bismuth, when used on osmium-fixed tissues without periodate oxidation, was reported to be specific for α-1,2 glycols (12). However, no differences were observed between diseased and healthy tissues in this study. Park (10) used this technique on leaves of Japanese pears treated with *Alternaria kikuchiana* toxin. The tissues in his experimental plants were doubly fixed in glutaraldehyde and osmic acid. The staining reaction was much more intense than those found in this study and is probably attributable to the reduction of bismuth by osmium present in the tissue. The problems introduced by osmium in both the silver methenamine and alkaline bismuth technique were discussed by others (1,6).

The Thiery technique showed no differences between healthy and victorin-treated roots. This stain is favored by many cytochemists because it produces numerous fine grains and visually pleasing images. However, it is not clear that it shows the total aldehyde content (3). The results of the present study suggest that the Thiery technique should only be used in combination with other methods when differences between treatments are sought. Victorin treatment may result in an increase in a particular class of oxidized sites detectable only with certain methods. Bayliss and Adams (2) reported that phenylhydrazines were more effective blocking agents than were semicarbazide or dimedone in the periodic acid-Schiff reaction on various animal tissues. In the present study, the opposite result was found. This may reflect differences in reactive groups.

Previous ultrastructure studies of victorin-treated tissues showed early changes associated with the cell wall-plasmalemma region (5,8). Cell wall lesions, which were observable after 3 hr in this study, were so named because their origin appeared to be at least partly from the cell wall (5). Park (10) suggested that amorphous materials between walls and plasma membranes of AK-toxin treated leaves also originate from the cell wall. The confirmation that wall changes are an early result of victorin treatment suggests that further research on the activation of host cell wall enzymes is warranted.

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