Pathological Changes in Ultrastructure: Effects of Victorin on Resistant Oat Roots

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ABSTRACT

Oat roots, resistant to victorin, were exposed for periods of 4, 10, and 20 hr to solutions which contained 200 units of victorin/ml. In roots exposed for 4 hr and fixed in $KMnO_4$, cortical cells had densely stained walls, hypersecretory Golgi dictyosomes with densely stained vesicles, and more or less parallel profiles of the endoplasmic reticulum. These features, which are not found in the cortex of nontreated roots, are similar to those described in susceptible roots exposed to much lower concentrations of victorin. In resistant roots treated with victorin for 4 hr, a few individual cells or isolated groups of 2-4 cells were severely disrupted, with only mitochondria recognizable as relatively intact organelles. These disrupted cells were similar to severely damaged exterior cells of victorin-treated susceptible roots. Resistant roots treated with victorin for 10 and 20 hr showed the same effects as those treated for 4 hr, except that some had unusually large Golgi vesicles and a few cells completely disrupted, with not even mitochondria recognizable. Cell wall lesions which are abundant in susceptible roots treated with victorin were not found in treated resistant roots. These results are consistent with physiological data, which indicate that at high concentrations, victorin induces in resistant tissues many of the changes found in susceptible tissues exposed to very low concentrations of this pathotoxin. Phytopathology 61:641-644.

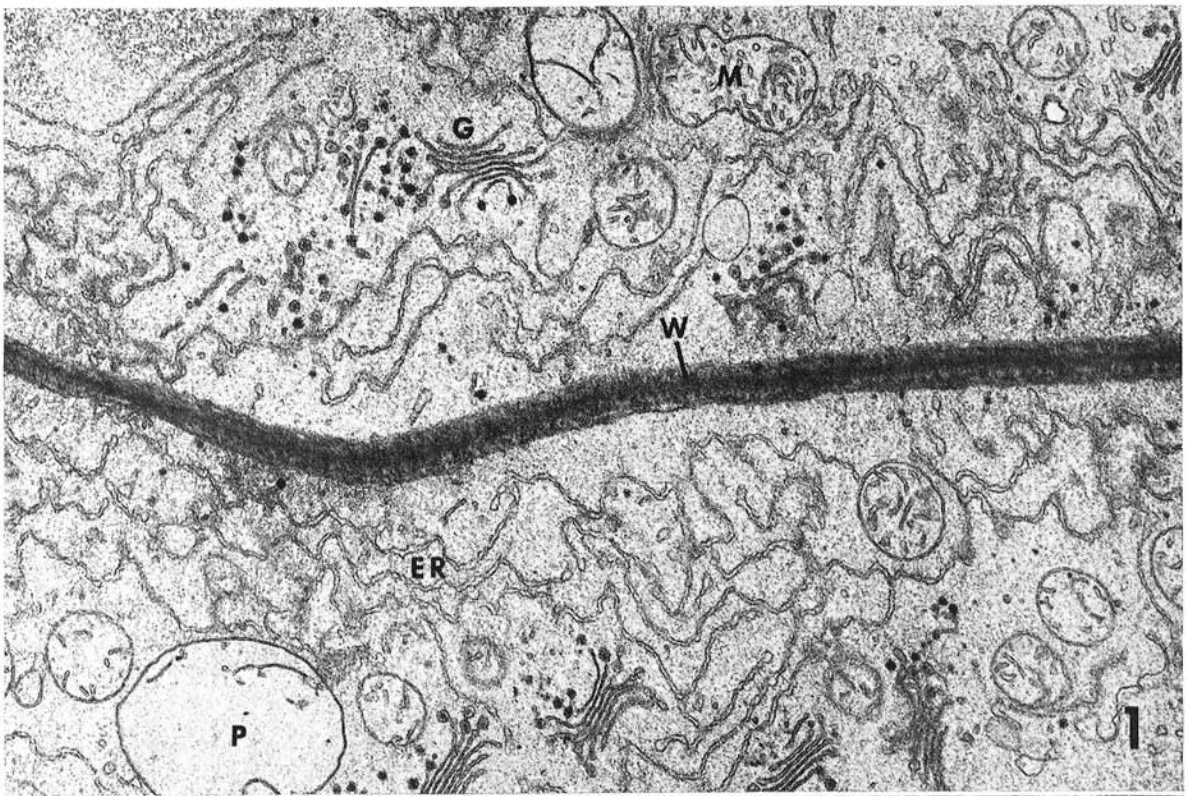
Additional key words: Helminthosporium victoriae.

Victorin, the pathotoxic product of *Helminthosporium victoriae* Meehan & Murphy, has been used extensively to study physiological changes which occur during disease development (8). The ultrastructural effects of victorin on cells of susceptible oat (*Avena sativa* L.) plants have been investigated as part of a long-term attempt to correlate pathological changes in function and structure in diseased plants (1, 2, 3, 4). When susceptible oat roots were exposed to victorin (0.001-10 units/ml for 1-24 hr) and fixed in $KMnO_4$, the earliest effects seen in epidermal and outer root cap cells were changes in the plasmalemma, which made its unit structure easier to resolve, and the formation of cell wall lesions which enlarged to form blisterlike structures between the cell wall and the protoplast (1, 4). In addition, in interior cortical cells of victorin-treated roots, cell walls became electron-dense, dictyosomes hypersecretory, and the endoplasmic reticulum arranged in roughly parallel profiles (1). Later effects included general disruption of all membrane systems, with mitochondrial membranes the last to become disorganized (1, 4). None of these effects was seen in resistant roots exposed to the same victorin treatments (1, 2, 4).

Although victorin is highly selective for oat cultivars susceptible to *H. victoriae,* resistant plants are not immune. Resistant tissues treated with victorin solutions containing 100-200 units/ml exhibit changes in permeability, respiration, transpiration, and isoperoxidase patterns similar to those found in susceptible plants treated with solutions containing 0.001 units/ml (5, 7). Since in previous ultrastructural investigations the highest concentration of victorin used was 10 units/ml, it seemed desirable to repeat this work with more concentrated victorin solutions, which are known to produce physiological changes in resistant tissues.

MATERIALS AND METHODS.—Methods for growing and treating roots and procedures for preparing material for electron microscopy were those described previously (1). The oat cultivars Compact (C.I. 8280) and a Victorgrain mutant (C.I.7418) were sources of resistant roots. Intact roots were exposed to victorin (200 units/ml) for 4, 10, or 20 hr. The victorin solution was the same refined preparation used in a previous study of physiological effects (7), and control roots were exposed to a deactivated solution. For each exposure period, at least two (in most cases three or more) treated and control roots of each cultivar were examined. Results within replications were consistent, and the pictures presented are typical. Roots were fixed in either aqueous $KMnO_4$ or phosphate buffered (pH 7.2) glutaraldehyde followed by osmium tetroxide ($Ga\text{-}OsO_4$ fixation), and sections were poststained in lead citrate as previously described (1). The magnification standard was a 28,800 line-diffraction grating replica.

RESULTS AND DISCUSSION.—*Effects and victorin on cell walls, Golgi apparatus, and endoplasmic reticulum.*—Three consistent effects were found in cortical cells of resistant roots exposed to victorin (200 units/ml) for 4 hr, then fixed in $KMnO_4$. Cell walls were electron-dense, dictyosomes were hypersecretory with densely stained vesicles, and the endoplasmic reticulum was arranged in roughly parallel profiles (Fig. 1). These same three effects are among the early changes found in cortical cells of victorin-treated susceptible roots (1). As was the case with susceptible roots, no increase in electron density of cell walls or Golgi vesicles was found when victorin-treated resistant roots were fixed in $Ga\text{-}OsO_4$. Hanchey et al. (1) pointed out that the densely stained cell walls and Golgi secretory products found in the cortex of victorin-treated roots fixed in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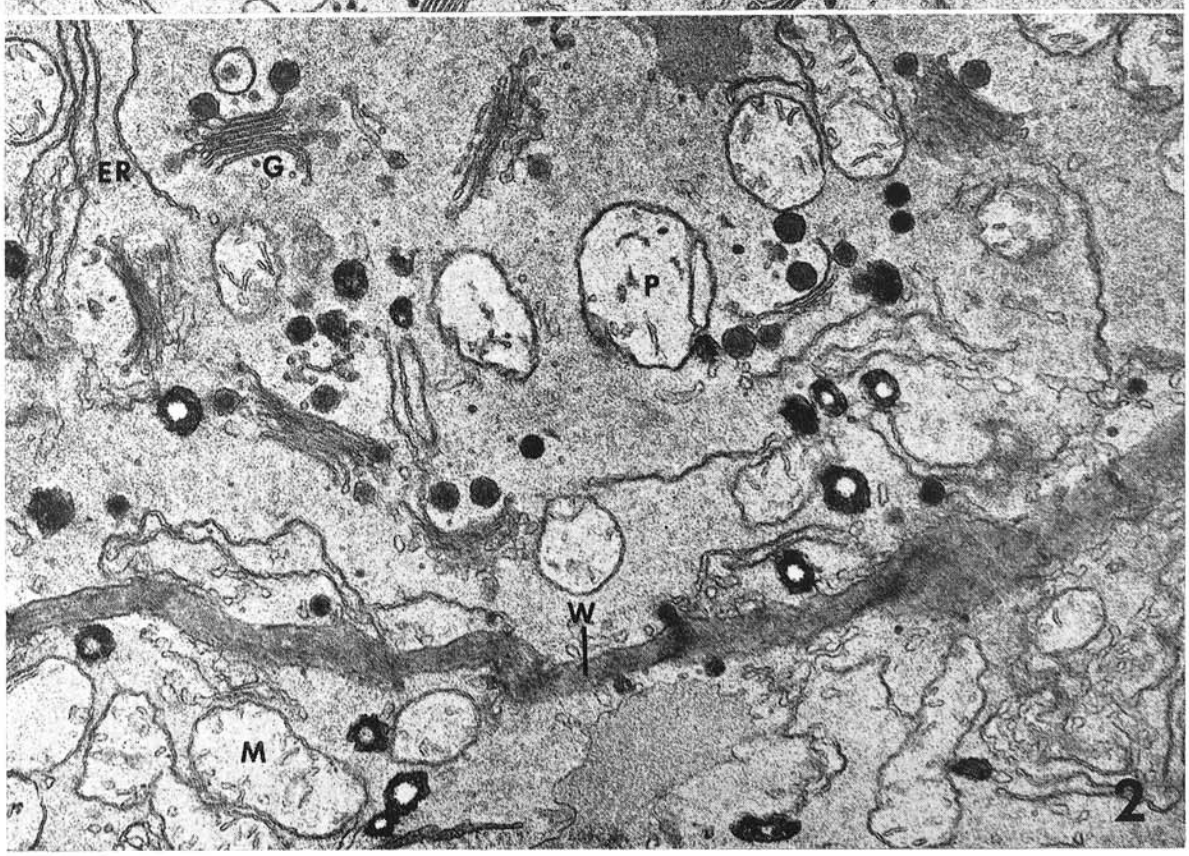
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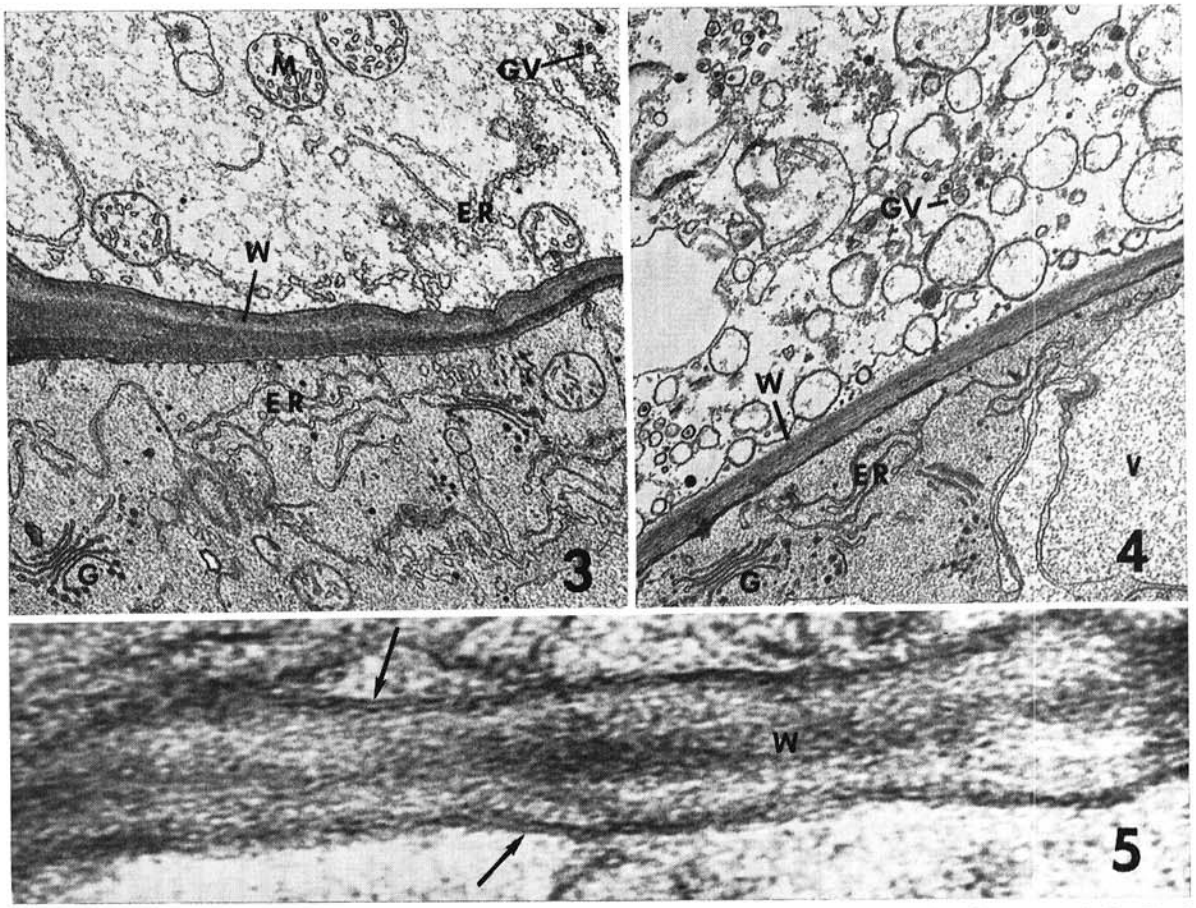

Fig. 3-5. Effects of victorin (200 units/ml), the pathotoxic product of *Helminthosporium victoriae,* on cortical cells of resistant oat roots. **3, 4)** Severely disrupted cells (above) adjacent to cells (below) which show only effects on cell walls (W), Golgi dictyosomes (G), and endoplasmic reticulum (ER). The disrupted cell in **3)** from a root treated with victorin for 4 hr has relatively intact mitochondria (M), structures which are probably Golgi vesicles (GV), and partially disrupted endoplasmic reticulum membranes; **4)** from a root treated for 20 hr, structures which resemble Golgi vesicles are the only identifiable organelles. (Both ×15,000) **5)** Portion of a cell wall from a root exposed to victorin for 10 hr. Arrows indicate regions where the unit structure of the plasmalemma can be seen. (×150,000)

$KMnO_4$ are remarkably similar to those of untreated epidermal and outer root cap cells, and that the failure of these structures to stain dark in Ga-OsO_4-fixed material suggests a change in some carbohydrate component.

Results with resistant roots exposed for 10 or 20 hr to victorin (200 units/ml) were similar to those after 4 hr, except that unusually large Golgi vesicles were present in approx one-third of cortical cells examined (Fig. 2). In cells exposed for 10 or 20 hr, 200 of the larger vesicles averaged 160 nm in diam. This was approx twice the diam of vesicles in roots treated for 4 hr, and considerably greater than that of vesicles in untreated epidermal cells which average 100 nm (1).

Effect of victorin on membranes.—Although the vast majority of cells in victorin-treated resistant roots showed only the effects illustrated in Fig. 1 and 2, a few severely damaged individual cells or isolated groups of 2-4 cells were found in every treated root examined. In roots treated for 4 hr, mitochondria were still recognizable in these damaged cells, and structures which resembled Golgi vesicles and disrupted endoplasmic reticulum profiles were present (Fig. 3). In damaged cells of roots treated for 10 or 20 hr, all membrane systems were disrupted and the only structures which could be even tentatively identified were Golgi vesicles (Fig. 4). The type of membrane disruption found in a few cells of resistant roots was remarkably similar to that found in severely damaged cells of victorin-treated susceptible roots (1, 4). The situation, however, was reversed, since in susceptible roots an occasional relatively undamaged cell was found surrounded by completely disrupted cells (1).

Among the early effects of victorin on susceptible

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Fig. 1-2. Effects of victorin (200 units/ml), the pathotoxic product of *Helminthosporium victoriae,* on cortical cells of resistant oat roots. **1)** Cells from roots treated with victorin for 4 hr. (×19,000) **2)** Cells from roots treated with victorin for 20 hr. (×25,000) Note the electron-dense cell walls (W), hypersecretory Golgi dictyosomes (G), and roughly parallel profiles of endoplasmic reticulum (ER). Mitochondria (M) and plastids (P) appear normal.

roots was a change in the plasmalemma which made its unit structure more easily resolved over long stretches (1). No such change was found in victorin-treated resistant roots. In fact, unit membrane structure was more difficult to resolve in victorin-treated than in untreated resistant roots, but this could have been a result of the increased electron denseness of cell walls in treated roots. When the unit structure of the plasmalemma could be resolved in victorin-treated roots, it was visible only over short distances, and no change in over-all width was detected (Fig. 5).

An extensive search failed to provide any evidence of an increase in cell wall lesions in victorin-treated resistant roots. In outer root cap cells where these structures are normal (1), cell wall lesions were found as frequently in nontreated as in treated resistant roots. In the root interior where they are not normal but are found in great abundance in victorin-treated susceptible roots (1), only one was found among more than a thousand cells examined in victorin-treated resistant roots. Although cell wall lesions and related structures may play a protective role for individual cells in susceptible reactions (3), such structures do not appear to be the basis for the ability of resistant oat roots to tolerate high concentrations of victorin.

In general, these ultrastructural results are consistent with physiological data which indicate that the initial effects of victorin are qualitatively but not quantitatively similar in both susceptible and resistant plants (6). Obviously, the changes in ultrastructure found in resistant plants do not result in irreversible or irreparable damage. After an initial period of inhibition, resistant roots maintained in victorin (200 units/ml) grow at least as fast as control roots (7). Resistant tissues treated with lower concentrations of victorin than that used in this study showed marked increases in losses of electrolytes (7). This indicates that changes in permeability can occur in the absence of any detectable change in plasmalemma ultrastructure. Possibly, changes in the plasmalemma found in previous work with susceptible roots (1, 4) are signs of irreversible damage.

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