Victorin-Induced Changes of Peroxidase Isoenzymes in Oats

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Supported by Public Health Service Grant No. EF-00319. Kentucky Agricultural Experiment Station Journal Series Paper No. 69-11-123.

Accepted for publication 10 October 1969.

ABSTRACT

Treatment of susceptible oat leaves with 0.2-20 units/ml of victorin, the pathotoxic product of *Helminthosporium victoriae,* induced quantitative changes in peroxidase isoenzymes. No changes were found in resistant tissue treated with the same concentrations of victorin. However, with increases in the victorin concentration to 200 units/ml, similar alterations were produced in resistant leaves. These results provide further evidence of the ability of resistant tissue to respond to victorin, and of the quantitative character of resistance to this toxin.

Victorin-induced changes were compared with those in naturally senescent, detached senescent, and mechanically injured leaves. Effects of victorin are not duplicated by senescence, but are similar to those caused by wounds. This suggests that victorin-induced changes in isoperoxidases may primarily result from a nonspecific response to injury. Phytopathology 60:467-471.

The nature of resistance to the highly specific pathogen, *Helminthosporium victoriae* Meehan & Murphy, and its pathotoxic product, victorin, has been a subject of disagreement (14, 15). The specificity of victorin has been attributed to inactivation of the toxin by resistant tissues (15), or to failure of resistant tissue to take up victorin because of a lack of toxin receptors (14). The concept of lack of receptors as the sole basis for resistance is incompatible with evidence that victorin is inactivated in resistant coleoptiles (20) and that resistant tissues treated with high concentrations of victorin show the same physiological responses as susceptible tissue treated with much lower concentrations (22).

Changes in isoenzymes as a result of infection have been reported for a number of plant diseases (1, 4, 5, 6, 12, 19). Such changes may play a role in metabolic regulation and in defense reactions of the hosts. However, the significance of altered isoenzyme patterns in diseased plants is difficult to assess because similar changes have been found in wounded or detached tissues (4, 5, 6, 12). Interpretations may also be complicated by new forms of enzymes contributed by the pathogen (1, 19). With victorin as the disease-inducing agent, this complication is avoided.

We have compared isoenzyme patterns from healthy oat leaves with those from leaves treated with victorin and with those from detached, senescent, and injured leaves. Our first objective was to determine if susceptible leaves treated with victorin at concentrations that produced disease symptoms would show changes in isoenzymes. If changes occurred, treatment of resistant leaves with much higher concentrations would provide a further test of the ability of victorin to elicit a response in resistant tissues. A final objective was to determine whether changes in isoenzymes caused by victorin resembled those brought about by senescence or injury. Peroxidase was chosen for this study because its electrophoretical pattern is sensitive and reflects changes caused by disease, aging, light, or treatment with growth regulators (16). A preliminary report of these studies has been published in abstract (13).

MATERIALS AND METHODS.—The susceptible oat (*Avena sativa* L.) cultivar Victorgrain 48-93 and the resistant cultivars C.I. 7418, Camellia, and Red Rust Proof were test plants. Seedlings were grown in a soil: vermiculite mixture (1:1) in a growth chamber with a 12-hr photoperiod (1,600 ft-c) at 23 C ± 1 C. First leaves of plants 10-14 days old were cut 1 cm above the leaf sheath, and the cut ends were immersed in 10 ml of the experimental solution. Victorin preparations that assayed 10,000 units/ml were refined as in previous work (10), and controls were exposed to deactivated preparations. Susceptible tissues were treated 4 hr with victorin and then transferred to water for 12 hr before extraction. Resistant tissues were left in the victorin or deactivated victorin solutions for 16 hr. During treatment, the leaves were under light (10,760 lux) continuously.

Ten-day-old plants were the source of tissue for experiments with roots. Roots were treated in petri dishes with 0.5 g of roots in 5 ml victorin solution for 4 hr, after which the roots were placed in water for 12 hr. Coleoptile segments, 3 cm long with the primary leaves removed, were obtained as previously described (20). Coleoptiles were allowed to take up toxin in small test tubes for 4 hr, and were then transferred to water for 12 hr. For experiments with detached senescent leaves, first leaves of 10-day-old plants were detached and left in tap water in the growth chamber for 3 days. First leaves of 21-day-old plants were the source of attached senescent leaves. For experiments on the effects of injury, leaves were wounded by gently compressing between bastard-cut metal files and left on wet filter paper for 3 days in petri dishes.

Tissues were ground in a chilled mortar with 0.1 M Tris [tris(hydroxymethyl)amino methane]-HCl sucrose buffer, pH 8.0 (18). Extracts were centrifuged for 1 hr at 20,000 *g*, 2 C. Protein content of supernatants was determined by the method of Lowry et al. (9). Samples containing 0.05 mg of protein/tube were used for disc electrophoretic separation (3) at pH 8.4 for separating gels and pH 6.3 for stacking gels. At least three separate enzyme preparations from three dif-

ferent batches of tissue were used in each test, and the protein profiles obtained in each test were all very similar.

Peroxidase visualization was achieved by Ornstein's method with benzidine-HCl and 0.01% H_2O_2 (2). After 30-min incubation in the substrate solution, the gels were photographed. Development with either benzidine-HCl or guaiacol as hydrogen donors resulted in similar isoperoxidase patterns. However, since benzidine gave sharper bands with a wide gradient of color intensity, the results obtained with this donor are presented.

Slight variations in distance from the origin to the front of gels occurred. Frequently, bands migrating very close to each other fused together. Therefore, instead of numbering bands, Rp values (relative position of bands) were used.

RESULTS.—*Isoperoxidases of oat tissues susceptible to victorin.*—The pattern of isoperoxidases from oat leaves is characterized by two groups of bands: a fast-moving group with one highly active band (Rp 0.9) and a more slowly migrating group with several active bands (Rp 0.2-0.5). In young susceptible oat tissue, two of the slowly migrating bands (Rp 0.25 and 0.35) decreased in activity after toxin treatment (Fig. 1-A, B). A group of bands at Rp 0.05-0.2 also decreased in activity, and some of these disappeared. Only one band at Rp 0.45 increased in activity. The magnitude of both decreases and increases in activity was proportional to the concentration of victorin solution (compare Fig. 1-B and Fig. 4-A). After treatment with victorin concentrations higher than 20 units/ml, the tissue became wilted and peroxidase bands were diffuse and low in activity. But no changes in activity were found in leaves treated for 4 hr with concentrations of victorin lower than 0.2 units/ml and extracted 12 hr later, nor when leaves were extracted immediately after 1-4 hr treatment with 2 or 20 units of victorin/ml. Changes entirely similar to those in Victorgrain were found when Fulgrain, another susceptible cultivar, was treated with victorin.

Isoperoxidases of oat tissues resistant to victorin.—Resistant tissues treated with victorin concentrations (0.2-20 units/ml) that produced changes in susceptible tissue showed no changes in peroxidase patterns, but when resistant tissues were treated with 200 units/ml of toxin, the activity of isoperoxidases was affected. The resistant cultivar C.I. 7418, which is an isogenic line of Victorgrain (21), has an isoperoxidase pattern identical to that of Victorgrain (Fig. 1-A, C). An increase at Rp 0.45 higher than that observed in susceptible tissue occurred when C.I. 7418 was treated with 200 units/ml of victorin (Fig. 1-B, D), but the decrease in activity found in susceptible tissue at Rp 0.05-4.0 was barely evident in this resistant cultivar.

Only a few peroxidase bands were detected in tissue from Camellia (Fig. 2-A). Victorin (200 units/ml) also induced in this tissue an increase in activity at approximately the same position where the increase occurred in Victorgrain and C.I. 7418 (Fig. 2-B). In Camellia, activity at Rp 0.05-0.40 was very low in controls, and little if any decrease in this area occurred after treatment with victorin.

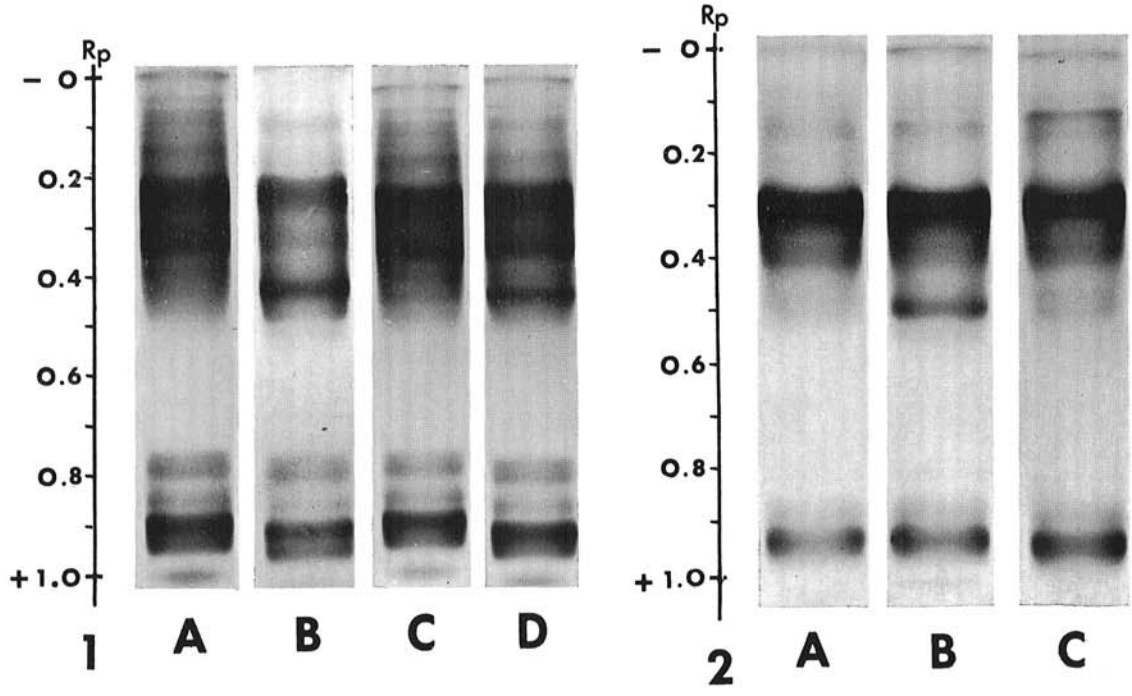
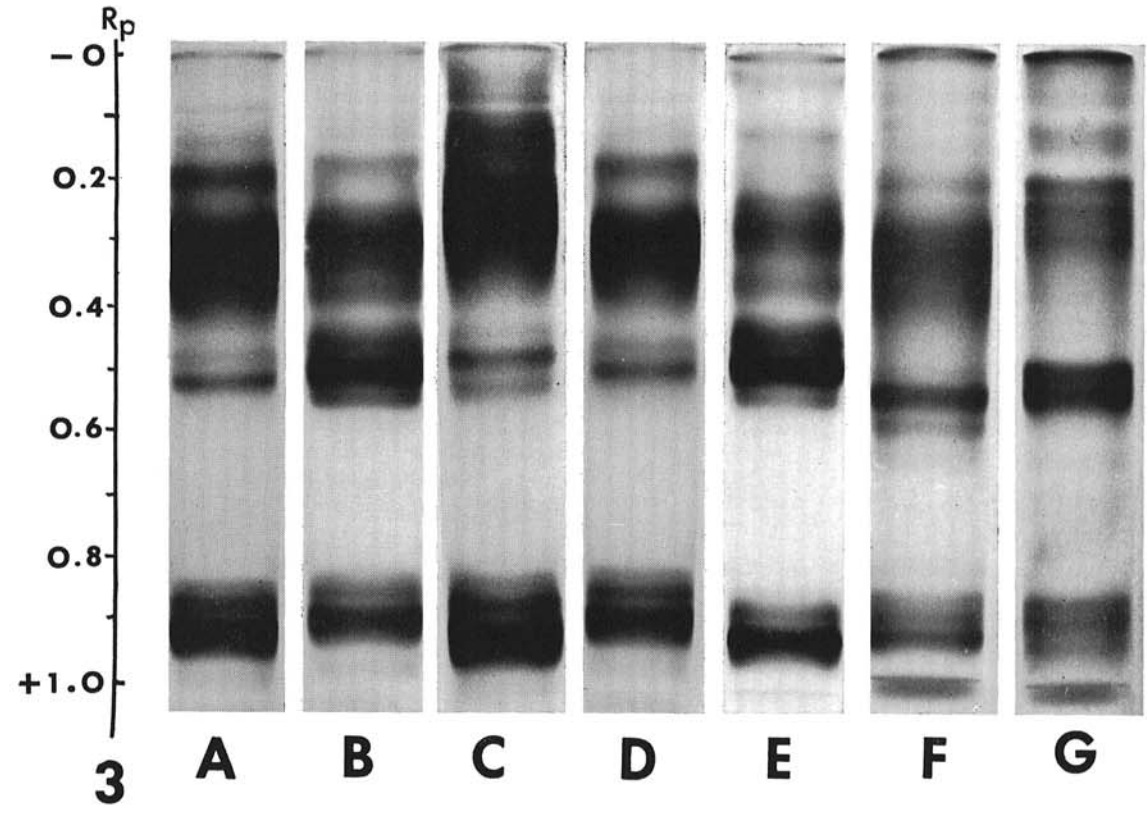


Fig. 1-2. **1)** Zymograms of peroxidase in the leaves of victorin-susceptible (Victorgrain 48-93) and resistant (CI 7418) oat cultivars. **A)** Victorgrain-control. **B)** Victorgrain-treated with 20 units/ml of victorin. **C)** CI 7418—control. **D)** CI 7418 treated with 200 units/ml of victorin. **2)** Zymograms of peroxidase in resistant oat leaves (cv. Camellia). **A)** Control. **B)** Treated with 200 units/ml of victorin. **C)** Detached senescent.



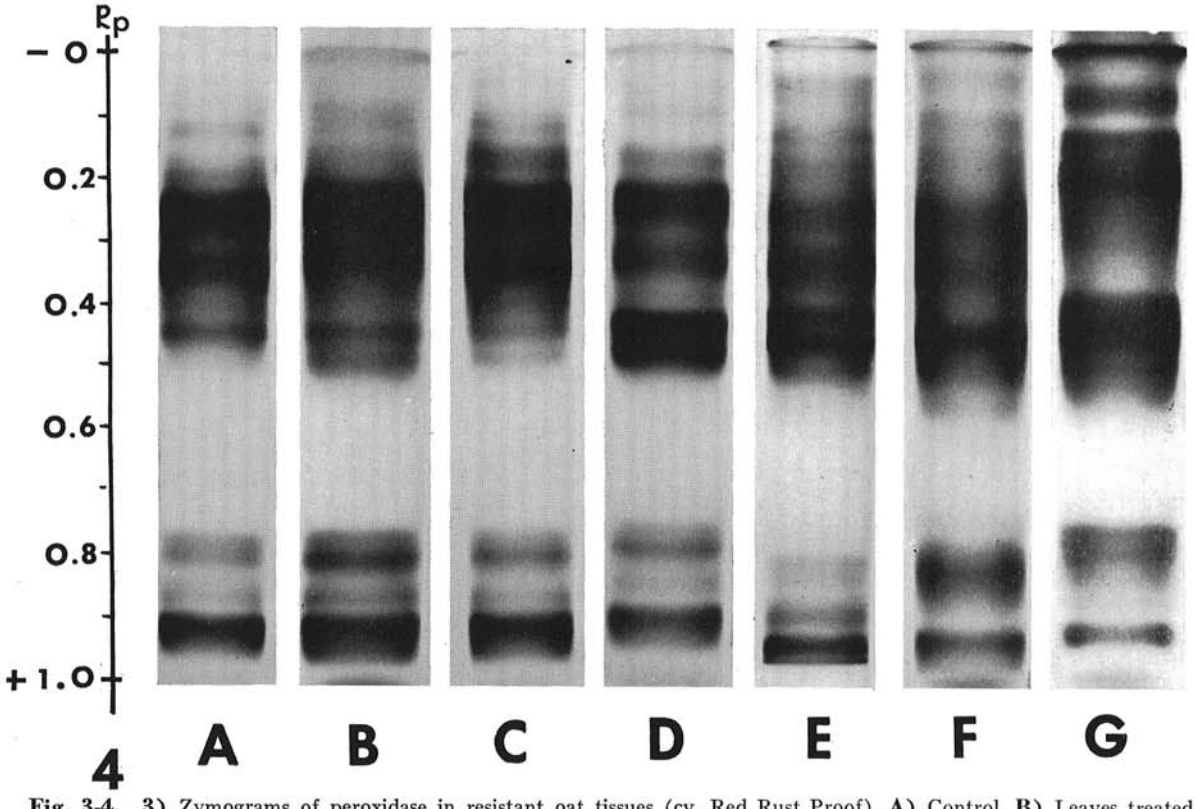


Fig. 3-4. **3)** Zymograms of peroxidase in resistant oat tissues (cv. Red Rust Proof). **A)** Control. **B)** Leaves treated with 200 units/ml of victorin. **C)** Detached senescent leaves. **D)** Attached senescent leaves. **E)** Mechanically injured leaves. **F)** Coleoptiles. **G)** Roots. **4)** Zymograms of peroxidase in susceptible oat tissue (cv. Victorgrain 48-93. **A)** Leaves treated with 2 units/ml of victorin. **B)** Detached senescent leaves. **C)** Attached senescent leaves. **D)** Detached senescent leaves treated with 0.2 units/ml of victorin. **E)** Mechanically injured leaves. **F)** Coleoptiles. **G)** Roots.

The most pronounced changes in isoperoxidases among all cultivars (susceptible and resistant) were in Red Rust Proof (Fig. 3). In this cultivar, a clear band at Rp 0.50 and a fainter one at Rp 0.45 were found in untreated tissue (Fig. 3-A). After treatment with victorin, a strong increase in activity occurred in a group of bands centered at Rp 0.50 (Fig. 3-B). As in Victorgrain, but to a lesser extent, activity at Rp 0.05-0.4 decreased.

Thus, treatment of two susceptible and three resistant cultivars with victorin resulted in a marked increase in activity of an isoperoxidase band at Rp 0.45. With the two susceptible cultivars, there was also a marked decrease in activity in a group of bands at Rp 0.05-0.40. The two resistant cultivars having appreciable activity in controls at Rp 0.05-0.40 showed only slightly reduced activity in this area after treatment, suggesting that processes leading to increased peroxidase activity are more sensitive to the effects of victorin than those resulting in decreased activity.

Isoperoxidase from senescent and wounded tissues.—When Victorgrain leaves were detached and allowed to become senescent over a 3-day period, slightly increased peroxidase activity was found in a group of bands at Rp 0.2-0.4, and a more marked increase in bands at Rp 0.45, 0.50, and 0.80 (Fig. 4-B). Changes identical with these were also found in detached leaves of the isogenic resistant line C.I. 7418. Senescence induced by detachment of Camellia leaves had little effect on isoperoxidases. Only a slight increase in activity at Rp 0.15 and at 0.50 was seen (Fig. 2-A, C). Detachment of Red Rust Proof leaves resulted in strong increases in activity in a group of bands at Rp 0.05-0.3 and a slight increase at Rp 0.50 (Fig. 3-C). The general increase in activity found in detached senescent leaves did not occur in leaves allowed to become senescent while remaining on the plant. Isozyme patterns from attached senescent leaves (Fig. 3-D, 4-C) were very similar, if not identical, to those from young control leaves (Fig. 1-A, 3-A).

When detached Victorgrain leaves were treated with low concentrations of victorin for 3 days, losses in activity similar to those caused by shorter exposures to higher victorin concentrations occurred in bands at Rp 0.05-0.4 (Fig. 4-A, D), but activity at Rp 0.45 and 0.50 was greatly increased over that caused by either detachment or victorin alone (Fig. 4-A, B, D). A similar marked increase in activity at Rp 0.45 and 0.50 was found with detached leaves which had been mechanically wounded (Fig. 3-E, 4-E).

Zymograms from coleoptiles and roots had more bands in the central region with high activity than those from leaves (Fig. 3-F, G, 4-F, G). This region includes the band at Rp 0.45; the activity of this band in untreated roots and coleoptiles was higher than that in victorin-treated leaves. This may account for the fact that treatment of roots and coleoptiles with victorin did not result in a detectable increase in activity at Rp 0.45. In agreement with previous observations of isoperoxidase differences among different plant organs (11, 17), patterns from roots (Fig. 3-G, 4-G) differed from those from coleoptiles (Fig. 3-F, 4-F); both differed from leaf patterns.

DISCUSSION.—Our results show that victorin induced similar changes in isoperoxidase patterns of susceptible and resistant tissues. We assume that in both tissues the same pathological shift in metabolism leads to the changes, and that only the concentration of the toxin necessary to induce them is different. These results are further evidence that resistance to victorin is a quantitative rather than a qualitative phenomenon.

Another question to be considered is the significance of peroxidase changes in diseased tissues. It has been suggested that increases in peroxidase activity or changes in isoperoxidases after infection may be related to disease resistance (8). In our case, there are several objections to this explanation. Concentrations of toxin which alter isoperoxidases and are highly toxic to susceptible tissues are without effect on resistant ones. The activity of the band which is increased by victorin is very high in healthy coleoptiles and roots, but these are very sensitive to the toxin. In other words, no correlation was found between the activity of this band and resistance. If a direct correlation between the activity of this band and the defense reaction existed, coleoptiles and roots should be more resistant to victorin than leaves. Although permeability and respiratory changes can be detected within minutes after exposure to victorin (7, 21), no changes in peroxidase patterns were found in susceptible tissue extracted immediately after 1-4 hr of treatment. Thus, isoperoxidase changes are not among the early effects caused by victorin, and probably occur too late to play an important role in resistance.

Our results do not support the hypothesis that isoenzyme changes in diseased plants merely reflect an acceleration of senescence. In all oat cultivars tested, victorin-treatment resulted in increased activity of a single band at Rp 0.45 with all other bands showing either a decrease or no change in activity. In contrast, detached senescent leaves had a general increase in activity of all bands, and in attached senescent leaves, patterns remained essentially unchanged. However, isoenzyme patterns from mechanically wounded detached leaves of Victorgrain were similar to those obtained when detached leaves were exposed to very low concentrations of victorin (Fig. 4-D, E). This was also true of mechanically wounded and victorin-treated Red Rust Proof leaves (Fig. 3-B, E). This suggests that changes induced by victorin may result primarily from a nonspecific response to injury.

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