An Assay for Helminthosporium victoriae Toxin Based on Induced Leakage of Electrolytes from Oat Tissue

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ABSTRACT

Rate of electrolyte loss from treated susceptible oat tissues was used to assay for the host-selective toxin of *Helminthosporium victoriae*. A direct relationship was found between toxin concn and rate of electrolyte loss, as measured by conductance of ambient solutions. Leakage rates increased with increases in toxin exposure time (1 to 120 min) and temp (5-24C). High concns (>10.0 μg/ml) induced no further leakage, indicating a saturation effect. Rates of leakage in response to subsaturating concns were linear for at least 3 hr and were used to quantify toxin by its effects. Standardized procedures are necessary for reproducible assays.

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The host-specific toxin from *Helminthosporium victoriae* Meehan & Murphy has a very early, and possibly a direct, effect on the plasma membrane, as shown by the immediate increase in loss of electrolytes and other materials from toxin-treated susceptible, but not from resistant, oat tissues (3). Toxin-induced leakage has been used for qualitative assays (1, 3, 5), but the available data were not adequate as a basis for quantitative work. We have re-examined the relationship between toxin concn and toxin-induced loss of electrolytes; the objective was the possible development of a precise assay.

MATERIALS AND METHODS.—Oat seedlings of cultivars resistant ('Rodney,' 'Garry,' and 'Clinton') or susceptible ('Park') to *H. victoriae* (HV) were grown in the laboratory as previously described (3). The toxin preparations were eluates from alumina columns, as described by Pringle and Braun (2).

Electrolyte leakage was determined by methods modified from those used in earlier work (1, 3, 5). Tissue samples (0.1 to 2.0 g) from the first true leaf of 6- to 21-day-old plants were enclosed in cheesecloth, vacuum-infiltrated for 10 min at 2 cm Hg, rinsed thoroughly in distilled water, placed in beakers containing 100 ml of toxin solution or water, and incubated for 1 to 4 h on a reciprocal shaker at 120 strokes/min. Then tissue samples were washed four or five times over a 10-min period, using 100 ml distilled water per wash. Each sample was finally placed in 50 ml distilled water (conductance, 1.0 to 1.5 μmho) and incubated on a shaker for up to 6 h. Conductance of the ambient solution was measured at intervals with a conductivity meter, using a dip-type electrode (K = 1.0). Triplicate samples in separate flasks were used for all treatments, and the procedure was modified as needed.

RESULTS AND DISCUSSION.—*Effect of toxin concentration.*—Electrolyte leakage from tissues increased as toxin concn was increased from 0.1 to 7.0 μg-ml, but no further increases in electrolyte leakage occurred above 10 μg toxin/ml. A plot of conductance vs. toxin concn yielded a hyperbolic curve (Fig. 1). A reciprocal plot of the same data gave a straight line. This relationship is of potential use in studies of toxin inhibitors (3).

When leaves were treated with a given concn of toxin, the rate of leakage was constant for at least 3 h (Fig. 2). This linear relationship further indicates that rates of loss can be determined accurately, and that induced leakage of electrolytes can be a basis for estimating toxin concns in solutions. The greatest leakage response per unit of this toxin preparation was in the 0.1-0.5 μg/ml range. Results were confirmed in several experiments with this same toxin preparation. The same relative results were obtained with three other partially purified toxin preparations, one of which completely inhibited growth of susceptible seedling roots at 0.001 μg/ml.

Temperature effects during the leaching period.—Previous data indicated that temp (5 to 37 C) during the brief time of toxin exposure had no effect on toxicity that was evident at a later time (4). We have now used a constant temp (22 C) during toxin exposure (2 h), and have varied the temp (5 to 24 C) during the subsequent leaching time. Electrolyte losses from leaves

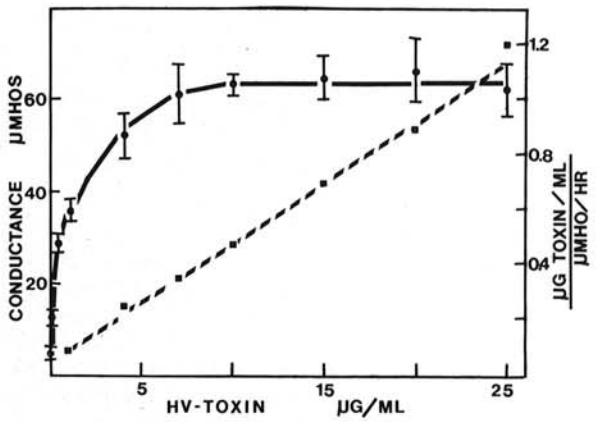

Fig. 1. Effects of HV-toxin at several concns on loss of electrolytes from oat leaves. Tissue samples (0.6 g each) were vacuum-infiltrated with water, incubated in toxin solution for 1 h, rinsed, and leached for 3 h in 50 ml distilled water for conductance measurements. Mean conductance values (μmhos/cm^3) and standard deviations are shown. Values for ambient solutions are plotted arithmetically (solid line, with scale to the left) and reciprocally (broken line, with scale to the right). The toxin solution completely inhibited susceptible seedling root growth at 0.16 μg/ml.

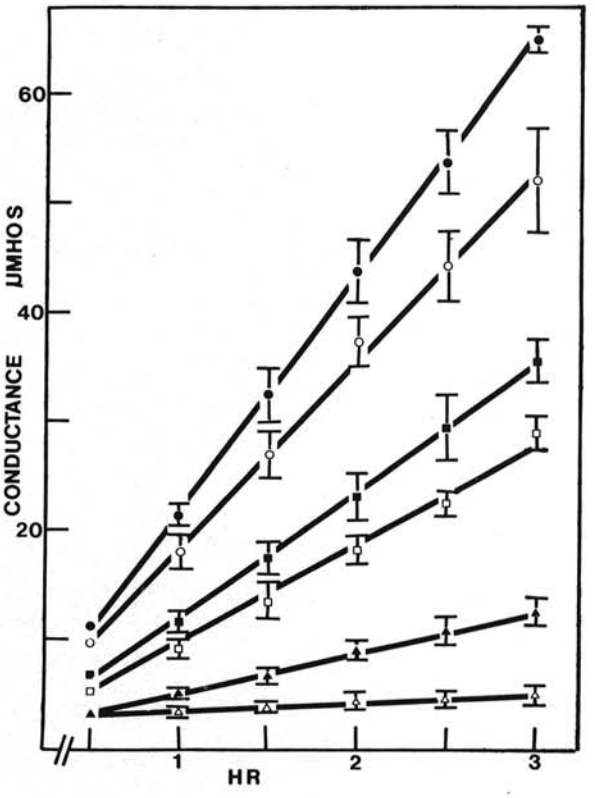

Fig. 2. Rates of electrolyte losses from oat leaves induced by *Helminthosporium victoriae* toxin at several concns. Data are from the experiment shown in Fig. 1. Toxin concns in μg/ml were 0.1; ▲; 0.5, □; 1.0, ■; 4.0, O; 10.0, ●; and O(control), △. Mean conductance and standard deviation are shown.

at 22-24 C were greater than losses from leaves at lower temp. For example, the conductance of ambient solutions of tissues leached at 12 C for 6 h was 58 μmhos, whereas comparable solutions from leaves leached at 22 C was 120 μmhos. The calculated Q_{10} values were often >2.0; others (5) have reported a Q_{10} value of 1.2 for toxin-induced losses from such tissues.

Effect of toxin exposure time.—Tissue samples (0.6-g each) were infiltrated in vacuo with water and incubated in toxin solutions. Conductance changes in ambient solutions of tissues treated with 1.0 μg toxin/ml were 2.5, 3.0, 6.5, 8.5, and 14.5 μmhos/h for toxin exposures of 1, 10, 30, 60, and 120 min, respectively. Conductance changed at a rate of 1.5 μmhos/h in control samples incubated in water. Comparable measurements for tissues treated with 10 μg toxin/ml were 8.4, 13.7, 20.0, 25.0, and 21.7 μmhos/h. Thus, rate of leakage increased with exposure time, provided subsaturating concns of toxin were used.

These data show several characteristics not evident in previously published data: (i) leakage induced by HV toxin has a saturation limit; (ii) a given concn of toxin causes leakage at a linear rate that is constant for 3 h or more; (iii) leakage is increased by increases in toxin concn over at least three orders of magnitude in the subsaturating range; (iv) definite rates of electrolyte leakage are set by each toxin exposure, and each fixed rate continues after tissues are removed from toxin.

Standardization of procedures.—Rates of toxin-induced losses from leaves increased with increase in age of assay seedlings, up to 18 days. On a common wt basis, larger samples (2.0 g) lost electrolytes at nearly double the rate for smaller samples (0.1 g). Toxin-induced leakage decreased with increases in size of leaf pieces (0.5 to 2.0 cm long) comprising the sample. Vacuum-infiltration with water did not increase the leakage over that of non-infiltrated controls. Vacuum-infiltration with toxin solutions resulted in more rapid electrolyte losses than those which occurred when leaves were floated on toxin solution, or were vacuum-infiltrated with water and incubated in toxin solution. Toxin-induced electrolyte loss was greater from seedlings grown with additional nutrients than from seedlings grown without additional nutrients. All these conditions must be standardized to achieve reproducible assays.

Electrolyte loss vs. root growth assays.—A toxin preparation was diluted 10^5 (0.42 μg/ml), 10^6, 10^7, 10^8, 10^9, and 10^{10} times for assay by the seedling root growth method. After 4 days, roots in the toxin dilution series averaged 4, 7, 14, 23, 48, and 41 mm, respectively, whereas susceptible control roots in water averaged 55 mm in length. The same toxin dilutions were used in electrolyte leakage assay with 0.6 g leaf samples from 16-day-old plants vacuum-infiltrated with water and incubated 4 h in toxin solutions. Conductance changes in ambient solutions of tissues exposed to toxin at 10^5, 10^6, and 10^7 dilutions, or to water, were 14, 7, 3, and 2 μmhos/h, respectively. The more dilute toxin solutions induced no significant increases in leakage. Essentially the same results were obtained in three different experiments. Thus, the sensitivity of the two assays was similar.

The 4-5 days needed for the root growth assay is too long for accurate assays of unstable, highly purified toxin. The assay based on toxin-induced loss of electrolytes can be completed 4-7 h after initial exposure of tissues. If all procedures are standardized, and if comparisons are made with known standards, the assay gives reliable estimates of the amounts of toxin in unknown solutions. Resistant tissue controls should be used in all cases.

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