Membrane Permeability in Plants: Changes Induced by Host-Specific Pathotoxins

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ABSTRACT

The effect of host-specific pathotoxins on the permeability of the plasma membrane and the tonoplast of host cells was determined using an ion efflux, compartmental analysis procedure. Toxin produced by *Helminthosporium victoriae* dramatically increased the permeability of both the plasma membrane and tonoplast of root cells in a susceptible cultivar (Park) of *Avena sativa*, but not in a resistant cultivar (Goodfield). A culture extract containing *H. maydis* race T toxin increased the permeability of plasma membranes of *Zea mays* leaf cells, but this effect was not host-specific. Under the experimental conditions used in this study, the *H. maydis* race T toxin, contained in the culture extract, had no effect on tonoplast permeability of host cells.

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Helminthosporium victoriae Meehan & Murphy, a root- and leaf-invading pathogen of oats, produces a toxin (victorin) which reportedly alters the permeability of plasma membranes in susceptible, but not in resistant, plants (13, 15, 16, 17, 18). It has also been suggested that the toxin produced by the fungus responsible for southern corn leaf blight, *H. maydis* Niskado & Miyake race T (HM-T toxin), may also alter membrane permeability (6).

The permeability of both the plasma membrane and tonoplast can be determined using an ion efflux, compartmental analysis procedure (3, 8, 10, 11). We have used this method to evaluate the effects of the two toxins on membrane permeability in plants which are resistant or susceptible to the respective fungi.

MATERIALS AND METHODS.—*Fungal toxins.*—Semipurified *Helminthosporium victoriae* toxin (victorin), provided by R. P. Scheffer of Michigan State University, East Lansing, was diluted 1:1,000 with water prior to use (12). A 1:1,000,000 dilution was sufficiently concentrated to kill susceptible oats, whereas a 1:40 dilution had no effect on tolerant oats (R. P. Scheffer, *personal communication*).

The semipurified culture extract containing *H. maydis* race T toxin, provided by A. L. Hooker and S. M. Lim of the University of Illinois, Urbana, was diluted 1:30 with water prior to use. This concentration of toxin prevented germination of susceptible but not resistant corn (A. L. Hooker, *personal communication*). The toxin preparation was the same one used by Miller & Koeppe (9).

Plants.—Cultivars of *Avena sativa* used in the present study were Goodfield (resistant to victorin) and Park (susceptible to victorin). The oats were grown in the dark for 4 days over aerated solutions of 1 mM $CaSO_4$ as described previously (5). The hybrids of *Zea mays*, obtained from Pfister Associated Growers of Aurora, Ill., were SX29-N (normal male fertile cytoplasm, resistant to HM-T toxin) and SX29-T (Texas type male sterile cytoplasm, susceptible to HM-T toxin). The corn was grown for 1 week in the greenhouse in aerated, one-sixth strength Hoagland's No. 1 nutrient solution (4).

Ion fluxes.—Permeability changes of membranes were determined by measurement of the rate of ^{86}Rb efflux from the tissue. Thus, two ion flux periods were involved; the first was for ^{86}Rb absorption and the second was for ^{86}Rb efflux from the tissue. Initially, roots (oats) or leaves (corn) were excised and cut into ca. 0.5-cm (length) segments and incubated in fresh culture solutions for 30-60 min to provide a wash prior to incubation in media containing ^{86}Rb. The oat root segments (2-3 g) were then incubated for 12 hr in 100 ml of aerated 1 mM KCl plus 1 mM $CaSO_4$ containing ^{86}Rb as the chloride salt (sp act of 5,000 cpm/μmole K^+). Corn leaf segments (2-3 g) were incubated for 12 hr in 100 ml of aerated, one-sixth strength Hoagland's No. 1 nutrient solution (4) containing ^{86}Rb (sp act of 5,000 cpm/μmole K^+). The temperature throughout the experiments was 25 ± 2 C. Continuous supplemental fluorescent light (ca. 500 ft-c) was provided for the corn leaves.

After the ^{86}Rb absorption phase of the experiment, the tissue (0.5 - 1.0 g) was placed into 20-ml plastic syringes (without plunger or needle) equipped with a rubber tube and pinch clamp. Five ml of nonradioactive solution, but otherwise identical to the absorption solution, were then added to the syringes containing the tissue (washout solutions). During the first 30 min of the efflux period, the solutions contained either the appropriate pathotoxin, or, as a control, equivalent amounts of noninoculated fungal growth medium. The growth medium for *H. victoriae* was not available for evaluation. The washout solutions were drained and replaced with fresh solutions periodically.

The ^{86}Rb content of the wash solutions (1 ml) and of the tissue (50-100 mg fresh wt) at the end of

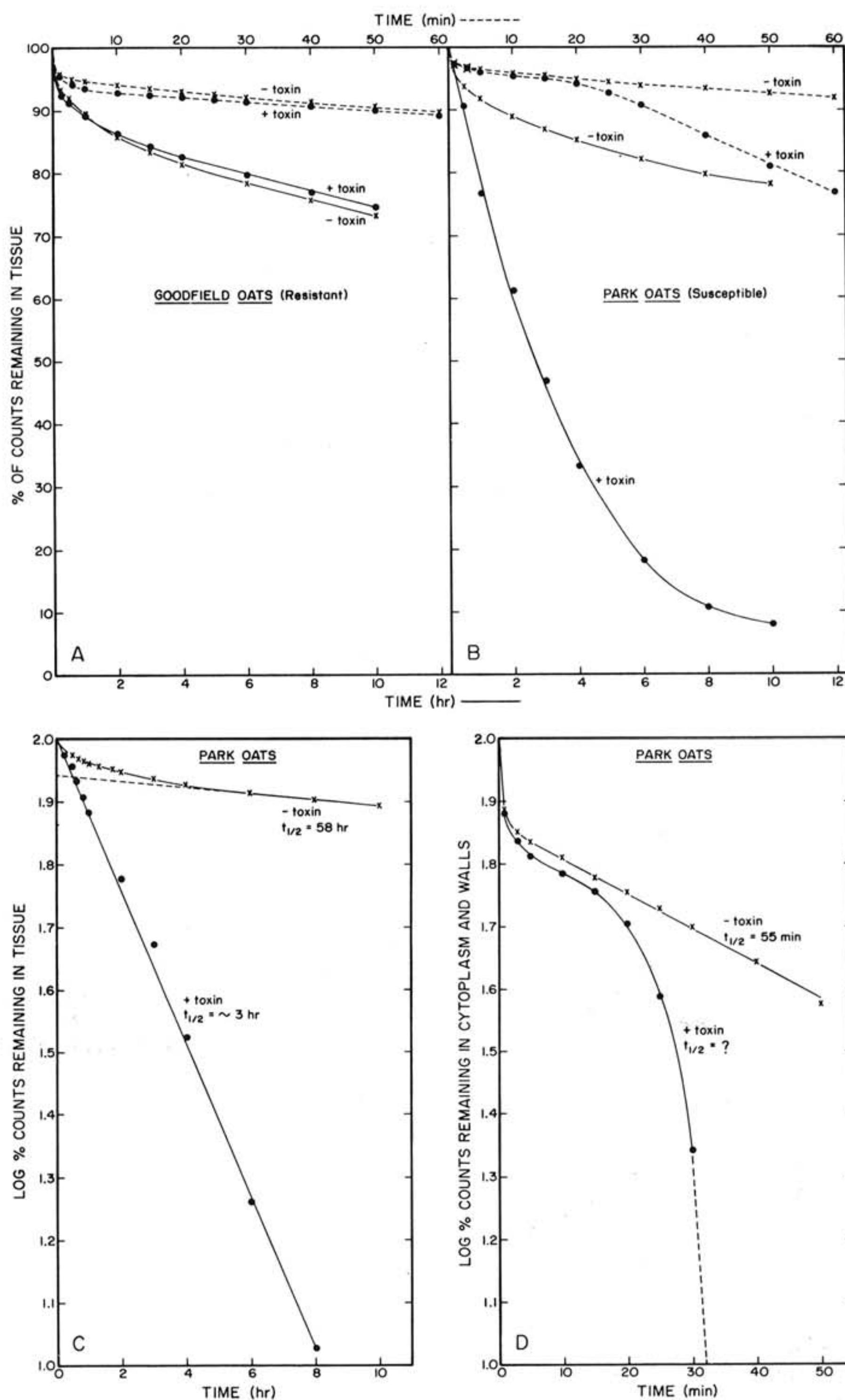

Fig. 1. Effect of *Helminthosporium victoriae* toxin on ^{86}Rb efflux from excised oat roots which are resistant (Goodfield) or susceptible (Park) to *H. victoriae.* **A, B)** Dashed lines are the ^{86}Rb losses during the first 60 min. Solid lines are for data from 15 min to 10 hr. **C)** Logarithmic losses of ^{86}Rb from entire tissue. **D)** Logarithmic losses of ^{86}Rb from tissue after subtraction of vacuolar content of ^{86}Rb.

the efflux period was determined directly with a liquid scintillation counter using 15 ml of scintillation fluid containing 60 g/liter Cabosil (2).

RESULTS.–*H. victoriae toxin*.–*Oat root experiments*.–The time course of ^{86}Rb loss, as a percentage of the counts remaining in the tissue, from both resistant and susceptible oats in the presence and absence of victorin, is shown in Fig. 1-A and B. Both short term (dashed lines) and long term (solid lines) losses are shown. The toxin had no observable effect on loss of ^{86}Rb from resistant (Goodfield) tissue (Fig. 1-A). However, the toxin caused a dramatic increase in the rate of ^{86}Rb loss from susceptible tissue (Fig. 1-B). About 20 min were required for the toxin to affect membrane permeability.

For assessment of the effect of the toxin on the loss of ^{86}Rb across the plasma membrane and tonoplast separately, the data of Fig. 1-B are replotted logarithmically in Fig. 1-C. A plot of this type yields a series of exponential curves, each of which represents the loss of radioisotope from a specific cell compartment (3, 8, 10, 11). The cell compartment contributing to the slow linear phase of ^{86}Rb loss is large (containing 80-90% of the ^{86}Rb in the present experiments) and is generally believed to be the vacuole (8, 10). This portion of the curve representing the loss of ^{86}Rb from vacuoles is, therefore, characteristic of the movement of ^{86}Rb across tonoplast membranes. A rate constant, k, can be calculated from the slope of the linear component of the curve; i.e., $k = \text{slope} \times 2.303$, and the half-time for ^{86}Rb loss from a particular compartment is equal to $0.693/k$. The half-time for ^{86}Rb loss from vacuoles of control roots was estimated to be 58 hr. In contrast, the half-time for toxin-treated roots was estimated to be only 3 hr. Thus, victorin dramatically increased the permeability of tonoplast membranes to ^{86}Rb in root cells of a susceptible variety of oats.

An estimate of the amount of ^{86}Rb in vacuoles at the beginning of the efflux period is obtained by extrapolating the linear component of the control curve (Fig. 1-C) to t = o. This value should be the same for both the control and toxin-treated roots, as they were treated identically prior to the beginning of efflux. If the ^{86}Rb in vacuoles is subtracted from the total ^{86}Rb in the tissue for the various time periods, the loss of ^{86}Rb from the remainder of the tissue (primarily cytoplasm and cell walls) is obtained. A logarithmic plot of these data is shown in Fig. 1-D. In the control tissue, a second linear phase of efflux, believed to represent the loss of ^{86}Rb from cytoplasm, was obtained. This loss, therefore, is characteristic of ion movement across plasma membranes (8, 10). The half-time for ^{86}Rb loss from cytoplasm in control roots was estimated to be 55 min. Although a corresponding half-time for toxin-treated tissue could not be determined since the rate of ^{86}Rb loss was nonlinear, it is obvious that the toxin affected plasma membrane permeability to ^{86}Rb. In fact, all the ^{86}Rb was lost from the cytoplasm in less than 35 min.

The rapid initial loss of ^{86}Rb from tissue is presumably from free space; i.e., cell walls and adhering surface film. The toxin does not have any appreciable effect on the loss of ^{86}Rb from this cell compartment.

The data clearly demonstrate that about 20 min were required for plasma membrane permeability to ^{86}Rb to be affected by victorin (Fig. 1-D). Also, on the basis of the early development of the linear component of ^{86}Rb loss from vacuoles in the toxin-treated roots (Fig. 1-C), it appears that tonoplast permeability was affected at approximately the same time. Thus, after 20 min the entire tissue loses ^{86}Rb as if only one compartment were present. Since the toxin was added only for the first 30 min of the efflux period, membrane damage was not only rapid, but irreversible.

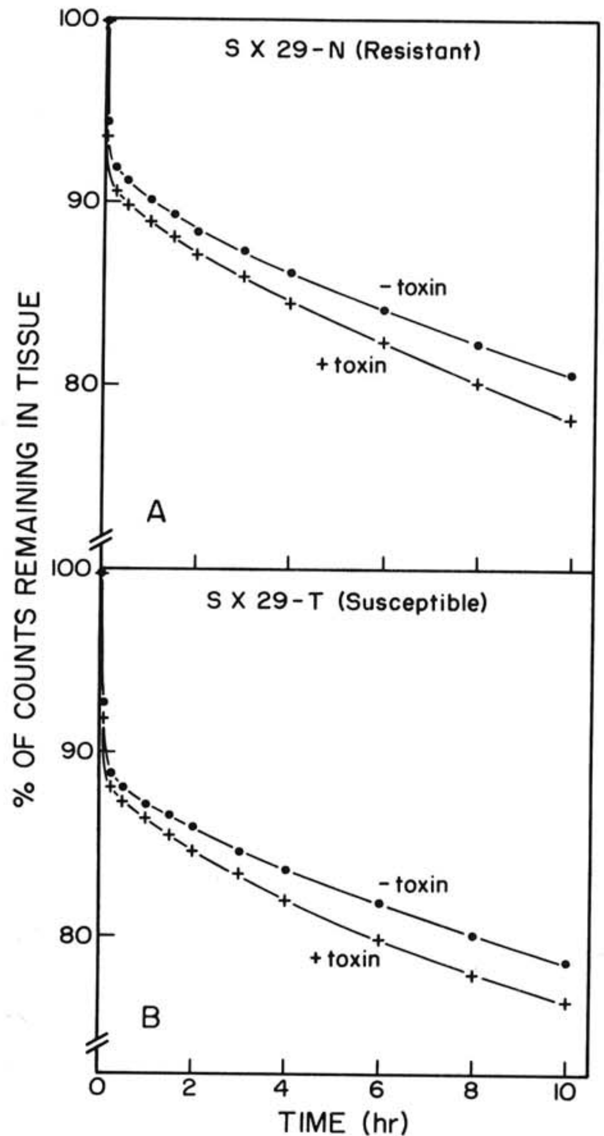

Fig. 2. Effect of *Helminthosporium maydis* toxin on ^{86}Rb efflux from corn leaf segments which are resistant (SX29-N) or susceptible (SX29-T) to *H. maydis*.

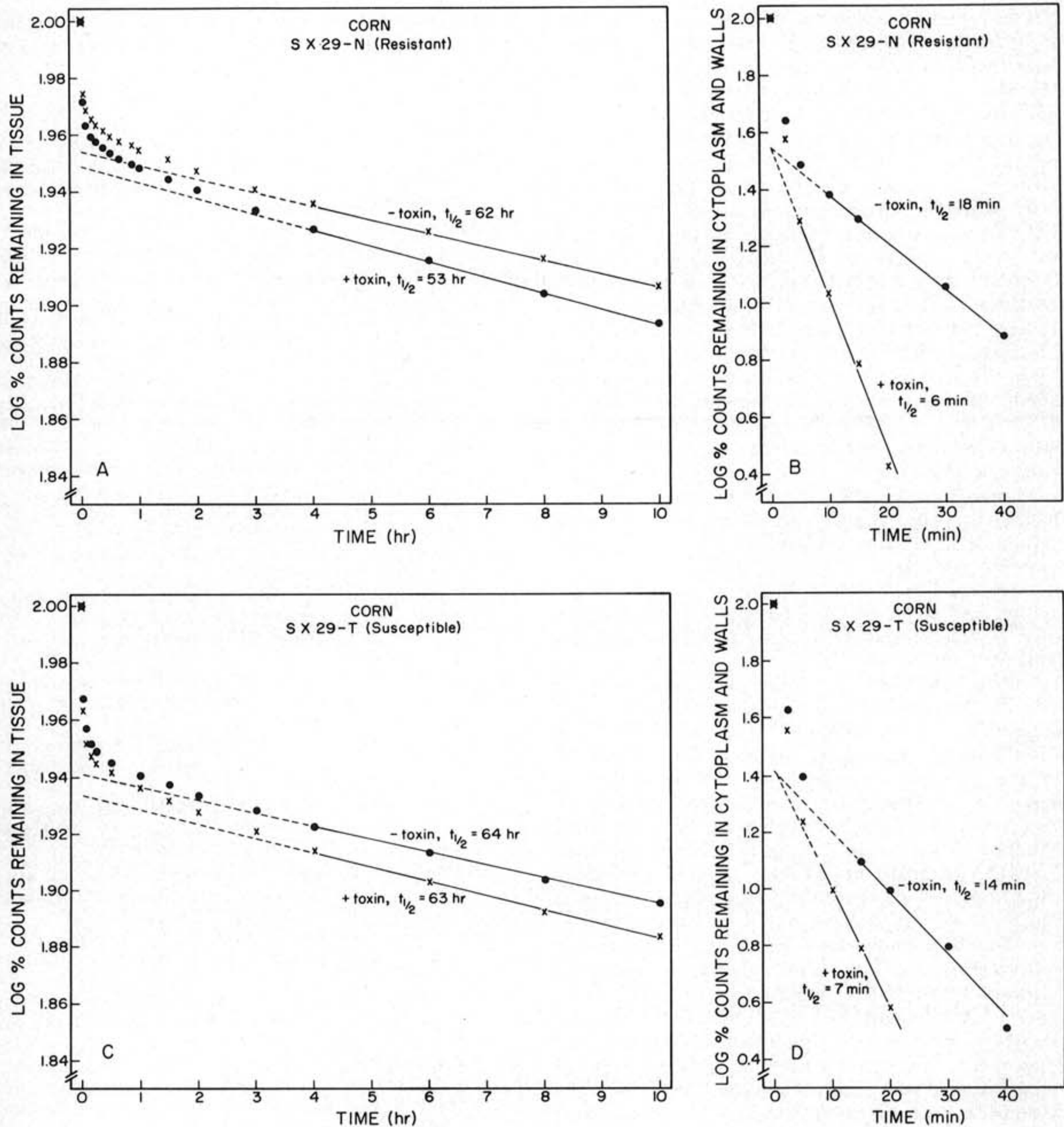

Fig. 3. A, C) Logarithmic losses of ^{86}Rb from entire tissue for the data shown in Fig. 2. **B, D)** Logarithmic losses of ^{86}Rb from tissue after subtraction of vacuolar content of ^{86}Rb.

H. maydis toxin.–Corn leaf experiments.–The effect of HM-T toxin on the loss of ^{86}Rb from leaf segments of both susceptible and resistant lines of corn is presented in Fig. 2-A and B. A slightly increased rate of ^{86}Rb loss from the tissue of both resistant and susceptible hybrids of corn resulted from the exposure to culture extracts containing HM-T toxin. Data are plotted logarithmically in Fig. 3 to determine the specific cellular compartment affected. Since half-times for ^{86}Rb loss from vacuoles of both susceptible and resistant tissues are similar, the toxin does not appear to have any effect on tonoplast permeability (Fig. 3-A and C). The HM-T toxin containing extract increases the permeability of plasma membranes (Fig. 3-B, and D), however, the effect is not host-specific; both resistant and susceptible tissues are affected similarly. On the basis of extrapolations to t = o for ^{86}Rb loss from the cytoplasm (Fig. 3-B, and D), it would appear that the toxin had a near instantaneous effect on plasma membranes.

DISCUSSION.–Wheeler & Black (17, 18) were the

first to report that victorin affected the permeability of susceptible host cells. This effect was ascribed by Samaddar & Scheffer (13) and Scheffer & Pringle (15, 16) to a primary lesion in plasma membranes. However, Samaddar & Scheffer (13) observed that the apparent free space of susceptible tissue was increased to ca. 90% after a 4-hr exposure to the toxin. This indicates that tonoplast membranes had been damaged, however, the authors (13) inferred that this was probably a secondary effect. They did, however, observe a significant increase in the apparent free space after only a 30-min exposure of the tissue to the toxin. On the basis of ultrastructural studies, Luke et al. (7) also indicated that lesions in tonoplast membranes after exposure to victorin were a secondary response. In our studies, both the plasma membrane and tonoplast permeability to ^{86}Rb was affected by exposure to victorin, and the effect on both membranes was apparent within 20-30 min. Although more rapid responses to the toxin have been reported (16, 18), the present studies suggest that either the plasma membrane and tonoplast contain sites equally sensitive to the toxin, or the tonoplast is damaged rapidly by a secondary product or reaction. The fact that plasma membranes would be the first to come into contact with the toxin, when applied exogenously, could give rise to its being damaged first.

It has not been established yet as to whether victorin damages cell membranes through direct interaction with a constituent of the membrane or as a result of an impairment of some other metabolic function which is necessary for maintenance of membrane function. Oxidative energy metabolism does not seem to be influenced, since isolated mitochondrial activities are not affected by the toxin (1, 14). An impairment of lipid or protein synthesis could lead to membrane derangements, but since studies of this type have not been conclusive (16) more definitive studies are needed.

H. maydis race T toxin had no effect on tonoplast permeability to ^{86}Rb in corn leaf cells under the experimental conditions used here. The culture extract containing HM-T toxin, when applied to leaf tissue, did increase the permeability of plasma membranes; however, this was the case for both susceptible and resistant hybrids of corn. Thus, damage to plasma membranes was probably due to some nonspecific toxic substance present in the culture extracts. One property reported for HM-T toxin which may play a role in disease development is its effect on mitochondrial activities. Miller & Koeppe (9) have shown, for example, that HM-T toxin eliminates respiratory control in mitochondria obtained from susceptible, but not from resistant, hybrids of corn. Thus, it would appear that the mechanism of action of *H. victoriae* and *H. maydis* toxins is quite different.

The ion efflux and compartmental analysis procedure described here provides a simple way for evaluating plasma membrane and tonoplast permeability. It should be a useful method for examining the effects of other pathotoxins or additives suspected of having an effect on membranes.

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