Leakage from Corn Tissues Induced by Helminthosporium maydis Race T Toxin

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ABSTRACT

Toxin produced by *Helminthosporium maydis* race T is host-specific in increasing leakage of electrolytes, but non-host-specific in increasing leakage of carbohydrates from root and leaf tissue of corn. The observed rate of leakage is dependent upon temperature and time of exposure to toxin. The toxin preparation contains at least three active components.

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Corn plants containing Texas and certain related types of male-sterile cytoplasm (Tcms) are very susceptible to *Helminthosporium maydis* Nisikado & Miyake race T, the fungus responsible for the Southern corn leaf blight epidemic of 1970 in the United States (3). Susceptibility of Tcms corn plants has been attributed to a toxin produced by *H. maydis* race T that has specific activity against plants containing susceptible cytoplasm (3,4,8,9). Unpurified culture filtrates of race T tested on Tcms and normal (N) cytoplasm plants preferentially inhibited seedling root growth (3,4,9), induced leaf necrosis (8), and caused young-leaf chlorosis (8) of Tcms plants. Mitochondria isolated from Tcms plants swelled rapidly and had reduced respiratory rates and lower ADP:O ratios after treatment with crude culture filtrates. Mitochondria from N plants were not affected(5).

During the first 18 hr of fungal ingress into leaves and within 8 hr after injection of a partially purified toxin preparation, we observed that leaves on Tcms plants developed a water-soaked appearance. This suggested that membrane permeability was affected early in the disease syndrome, resulting in leakage of cellular fluids. Changes in membrane permeability are induced by the host-specific toxins produced by *H. victoriae, H. carbonum*, and *Periconia circinata* in their respective susceptible hosts: oats, corn and sorghum (7). We have shown that toxin from *H. maydis* race T is selective in causing leakage of electrolytes from susceptible corn tissue.

MATERIALS AND METHODS.—The fungus was grown, and toxin was extracted from agar and mycelial mats with methanol, as described by Turner & Martinson (8). Methanol was removed under reduced pressure, and the aqueous suspension was reduced to one-tenth of the original culture volume and extracted with an equal volume of ethyl acetate. The extraction was repeated four times, and the ethyl acetate fractions were combined and evaporated to dryness under reduced pressure, leaving a brown, oily residue. This residue was then extracted with water and made up to a volume equal to one-tenth that of the starting material and stored at -20 C. For experimental purposes, this toxin preparation was diluted 100-fold in water and had a conductance of 0.7 μmhos greater than distilled water.

Chromatography of ethyl acetate-soluble toxin was done on Whatman No. 3 paper, by the ascending method, using a solvent of ethyl acetate:methanol:water (4:1:1). Chromatograms were cut into horizontal strips corresponding to 0.05 R_F, eluted with water, and the eluates were assayed for toxin activity with the plant whorl assay of Turner & Martinson (8).

All experiments were done by using 30 ml of water or toxin solution in a 50-ml beaker for roots and a 125-ml flask for leaves per replicate sample. Root leakage was measured on 2.5-cm radicle tips (20 radicles/30 ml) from 5-day-old seedlings. Excised radicles were rinsed in distilled water for 2 hr before the start of experiments. Radicles were immersed in toxin solution for the entire experiment. Corn varieties used in root studies were race T-susceptible B37TcmsHt X B14AHt, and race T-resistant B37Ht X B14AHt. Leakage from leaves was measured on 1-cm leaf pieces (1 g/30 ml) from 12-day-old seedlings of race T-susceptible WF9Tcms and race T-resistant WF9 corn. Leaf tissue was placed in either toxin or distilled water and vacuum infiltrated to start experiments. Rates of leakage were determined by measurements on the ambient solutions.

Unless otherwise noted, experiments were done with a single toxin preparation, using a stationary water bath at a temperature of 32 C for roots, and on a reciprocal shaker at 25 C for leaves; measurements were made on the ambient solutions. Conductance was measured with a Yellow Springs conductivity bridge (Model 31) with conductivity cell 3403 (K = 1.0). Total carbohydrate was determined by the method of Dubois et al. (2), standardized for maltose. Rate of leakage was calculated by the formula: R =

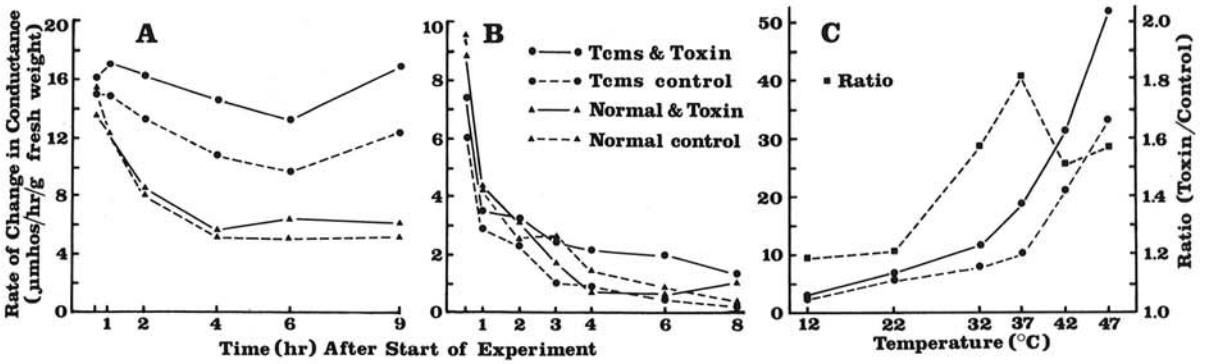

Fig. 1 A,B,C. Time course of change in rate of electrolyte loss from roots **A)** and leaves **B)** of corn containing either Texas male-sterile cytoplasm (Tcms) or normal corn as affected by toxin. Points are located at the end of the time periods for which rates were determined. **C).** Rate of electrolyte loss from radicles of Tcms corn in the presence and absence of toxin and a ratio of rates (toxin/control) at various temperatures. Values indicate the rate of electrolyte loss during the period from 2 to 4 hours after toxin exposure.

$(C_{t2} - C_{t1})/(T \times W)$ where R = rate of leakage, C_{t1} and C_{t2} = conductance or carbohydrate concentration at the beginning (t1) and end (t2) of the time period, T = elapsed time in hours between t1 and t2, and W = fresh weight of tissue in grams. Total soluble carbohydrate was determined by correcting for volume at t2.

RESULTS.—Bioassays of chromatogram eluates showed three well separated regions of toxin activity; one at the origin, one at the solvent front and one at approximately R_F 0.40 to 0.50. Quantitative data on the relative activities of these three components is not available, and it is not known if the activities are those of single compounds or if there may be more than three active compounds.

Figures 1-A and 1-B illustrate the time course of electrolyte leakage as affected by toxin in both roots (1-A) and leaves (1-B) from Tcms and N plants. No significant differences in conductance (5% level) were observed between control and toxin-treated N tissues during these experiments. Rates of leakage from toxin-treated Tcms tissues were higher than rates from untreated tissues at all time periods, and these differences were statistically significant at all time periods greater than 1 hr after the beginning of the experiments. Relative differences between rates of leakage from toxin-treated and control tissues increased until 2 to 4 hr after the experiments were started, after which they remained nearly constant. Therefore, rate differences between control and toxin-treated tissues are dependent upon the time period for which conductivity is measured. In experiments with a more active toxin preparation, we observed statistically significant increases in electrolyte loss from radicles as early as 10 min after placing radicles in toxin.

The effect of temperature on electrolyte leakage from Tcms radicles is shown in Fig. 1-C. Both the rate of leakage and the difference between control and toxin-treated tissues is increased by an increase in temperature over the entire temperature range studied (12-47 C); the ratio of rates (toxin/control), however, reaches a maximum at 37 C. Differences between toxin-treated and control tissues were significant at all temperatures greater than 12 C. The decrease in the ratio above 37 C corresponds closely to a rapid increase in the rate of electrolyte loss from both toxin-treated and control tissues. Extrapolation of curves representing rates of leakage (Fig. 1-C) indicates that a change in slope occurs at 35 - 36 C. Thermal damage or change in membrane structure may begin at this temperature.

Research on *H. victoriae* toxin has established that both electrolytes and nonionic substances may be lost from tissues in the presence of a toxin (1). Loss of carbohydrates as well as of electrolytes was induced by *H. maydis* race T toxin (Table 1). As in previous experiments, the only statistically significant differences in electrolyte leakage were between control and toxin-treated Tcms tissues. When relative rates of carbohydrate leakage were calculated,

TABLE 1. Rates of leakage of electrolytes and carbohydrates from corn radicles and leaves containing either Texas male-sterile cytoplasm (T) or normal cytoplasm (N) in the presence and absence of toxin

	Rate of leakage					
	Electrolyte			Total carbohydrate		
Cytoplasm and tissue	μmhos/hr/g Control		Toxin	μg/hr/g Control		Toxin
N-roots	18.9		19.6	32	*	115
N-leaves	0.9		0.7	13	*	35
T-roots	20.7	*[a]	26.1	97	*	309
T-leaves	1.1	*	2.6	15	*	56

[a]An asterisk (*) between two values indicates a significant difference at the 5% level.

however, a three-fold increase in rate of leakage due to toxin was observed in both Tcms and N tissues. Amino acid concentrations in the ambient solutions determined by the ninhydrin method of Moore & Stein (6) were below the reliable limit of the method (0.02 μmole). Thus, differences in toxin-induced leakage between Tcms and N tissues depended upon the type of substance measured.

To determine if tissues are able to recover from toxin treatment and return to a normal rate of electrolyte loss, we vacuum infiltrated and held leaf segments in toxin for 3 hr. They were then rinsed repeatedly with distilled water for 1 hr and were placed in flasks of distilled water for conductance measurements. Toxin-treated Tcms tissues continued to show a higher rate of electrolyte loss (7.1 μmhos/hr/g fresh weight) at 4 hr after being removed from toxin solution than did untreated leaves (0.7 μmhos/hr/g fresh weight). This indicated toxin-induced leakage was irreversible or that toxin was tightly bound in Tcms tissue and was not removed by repeated rinsing. Again, N leaves showed no toxin-induced loss of electrolytes.

DISCUSSION.—*H. maydis* race T toxin is able to induce rapidly an increase in the rate of electrolyte loss from susceptible corn tissues, as do several other host-specific toxins on their respective graminaceous hosts. However, this toxin may not be entirely host-specific in its effects on corn, as demonstrated by the fact that it increases the rate of carbohydrate loss from both Tcms and N tissues. The toxin is apparently entirely host-specific in producing macroscopic symptoms. In preliminary experiments, using the plant whorl assay of Turner & Martinson (8), a preparation 100 times more concentrated than that used in these studies gave no reaction in N plants, whereas one 50 times more dilute gave a positive chlorotic streaking reaction in Tcms plants. Similarly, Miller & Koeppe (5) reported the toxin to be completely differential in its effects on mitochondria from N and Tcms plants. The effect of the toxin on leakage of electrolytes and carbohydrates suggests the plasma membrane may be the primary site of action of the toxin, but the leakage could also be secondary to an effect of the toxin on mitochondria or other sites within the cell.

Our isolate of *H. maydis* produces at least three compounds in culture which are able to induce chlorotic streaking in susceptible plants. The toxin preparation used in the studies on leakage was a mixture of these compounds, and we are unable to say if one or more of them is responsible for the leakage phenomena observed.

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