

2. Effects of the toxin of Helminthosporium maydis on cell membrane permeability.

Corn hybrids having the Texas male-sterile (Tcms) cytoplasm are more susceptible to southern corn leaf blight than are those having the normal male-fertile cytoplasm (N-cmf). Since diseased plants often display increased membrane permeability, the following experiment was undertaken in an effort to assess the effects of the toxin of the causal organism of the blight, Helminthosporium maydis Nisikado and Miyake, race T, on the cell membranes of susceptible plants.

An isolate of H. maydis, race T, was obtained from Dr. O. D. Morgan, Department of Botany, University of Maryland, College Park, Md. After inoculation into 500 ml lots of modified Fries' medium, the fungus was allowed to grow at room temperature for two weeks. The culture was harvested by filtration through Miracloth, after which the pH was raised to 7.0. Boiling for 5 minutes to kill spores was followed by freezing, and the culture filtrate was stored in the freezer until used.

Since earlier work in our laboratory had shown that effects of the crude culture filtrate were similar to those of partially purified toxin, the preparation of which requires many additional hours, the crude culture filtrate was used in the investigations.

Cut stem ends of the shoots of 13-day-old corn plants carrying either T-cms cytoplasm or N-cmf cytoplasm were placed in crude culture filtrate at 0° for 2 hours. Controls were placed in glass-distilled water. After toxin uptake, 1-gram samples of leaf tissue, cut into pieces 1-2 cm in length, were washed 5 times in glass-distilled water and transferred to 100 ml Ehrlenmeyer flasks which were then shaken at the rate of 110 strokes per minute at 25° C. Ten ml aliquots of the ambient solution were withdrawn at the end of 4, 8, 12, and 16 hours.

These samples were then analyzed, using a Perkin-Elmer 303 Atomic Absorption Spectrophotometer, for K^+ , Na^+ , Ca^{++} , and Mg^{++} .

The results, shown in Tables 1, 2, 3 and 4, indicate increased loss of all 4 cations from susceptible tissue as compared with loss from the controls and from the toxin-treated resistant tissue. We conclude from these preliminary data that one of the specific effects of the fungal toxin is the damage of cell membranes of lines carrying T-cms cytoplasm but not

N-cmf lines. Current investigation is directed at 1) defining the molecular damage and 2) characterization of the cell membranes from T-cms and N-cmf lines for differences in protein and/or lipid quality and/or quantity.

Table 1

Cytoplasm-Treatment	Leaching Time (hrs)			
	4	8	12	16
T-toxin	1.4*	1.6	1.7	2.8
T-water	1.2	1.2**	1.1	1.1
N-toxin	1.1	1.2	1.2	1.2
N-water	.9	1.2	1.2	1.2

*Numbers are ppm K^+ in leachate; mean of five determinations

**Mean of four determinations

Table 2

Cytoplasm-Treatment	Leaching Time (hrs)			
	4	8	12	16
T-toxin	25*	25	26**	29
T-water	26	24**	23	23
N-toxin	29	26**	20	21
N-water	26	29	26	20

*Numbers are ppm Na^+ in leachate; mean of five determinations

**Mean of four determinations

Table 3

Cytoplasm-Treatment	Leaching Time (hrs)			
	4	8	12	16
T-toxin	1.7*	2.4	3.3**	3.9
T-water	1.8	2.0**	2.5	2.5
N-toxin	2.4	3.6	2.5	2.3
N-water	1.9	2.6	2.5	2.3

*Numbers are ppm Ca^{++} in leachate; mean of five determinations

**Mean of four determinations

Table 4

Cytoplasm-Treatment	Leaching Time (hrs)			
	4	8	12	16
T-toxin	.24*	.23	.51**	.66
T-water	.16	.14**	.32	.33
N-toxin	.28	.34	.33	.29
N-water	.19	.35	.33	.35

*Numbers are ppm Mg⁺⁺ in leachate; mean of five determinations

**Mean of four determinations

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1. Two systems that transform a two-ranked spike into a four-ranked spike.

The eight-rowed ear of maize (four-ranked with paired spikelets) can be derived from the two-ranked spike (distichous) of teosinte and certain variants in maize by two different systems as follows:

1. Condensation alone in which a lack of internode elongation in the rachis forces a primordial slippage or twisting in order to spatially