Mitochondrial Heredity: A Determinant in the Toxic Response of Maize to the Insecticide Methomyl

Abstract. *Mitochondria isolated from etiolated Texas male-sterile (TMS) cytoplasm maize (*Zea mays L.*) seedlings were adversely affected by methomyl (Lannate, 90 wettable powder), while those isolated from normal-fertile seedlings were not. In a manner analogous to that reported for* Bipolaris *(Helminthosporium)* maydis *(race T) toxin, experiments with TMS mitochondria showed that 1 to 3 millimolar methomyl inhibited the state 4 oxidation rate of combined malate and pyruvate while stimulating that of succinate or exogenous reduced nicotinamide adenine dinucleotide. Similar concentrations of methomyl effected an inhibition of phosphorylation, an increase in the percentage of transmittance of light through mitochondrial suspensions, and a decrease in the density of the mitochondrial matrix. Methomyl (15 millimolar) had little effect on the physiological activity or ultrastructure of isolated normal-fertile mitochondria. These observations provide the opportunity to specifically assess the homogeneity, or lack of it, of a cytoplasmic heritable characteristic in a widely divergent group of higher plants.*

Extranuclear DNA associated with mitochondria controls a limited number of cytoplasmic characteristics (*1*). In plants, this cytoplasmic inheritance includes male sterility in a number of species (*2*), and susceptibility to the fungus *Bipolaris* (Helminthosporium) *maydis* (race T) in maize (*3*). Whether the same genome is responsible for male sterility and *B. maydis* susceptibility is not known. However, mitochondria isolated from etiolated shoots of Texas male-sterile (TMS) cytoplasm show a number of adverse membrane effects when treated in vitro (*4*) and in vivo (*5*) by a toxin homogenate produced by *B. maydis* (race T). Mitochondria isolated from etiolated shoots with a comparable nuclear genome, but normal-fertile (N) cytoplasm, show little response to the toxin preparation except when the outer mitochondrial membrane has been removed (*6*).

While the differential toxin-induced response of mitochondria isolated from the two maize cytoplasmic types has presented an instance of a clearly different mitochondrial genome associated with a well-characterized nuclear genome, the precise nature of the interaction of toxin and mitochondria has been waiting for the characterization of the toxin itself. Karr *et al.* (*7*) isolated four toxins, which were characterized as having carbon skeletons similar to those of tetracyclic or pentacyclic triterpenoids. But further definition of the *B. maydis* (race T) toxin has not been forthcoming. Humaydan and Scott (*8*) observed that the relatively simple insecticide methomyl (Lannate, 90, wettable powder), when sprayed on whole plants produced different responses in TMS and N cytoplasms, thus providing a potential means for characterization of the biochemical differences in mitochondria isolated from TMS and N cytoplasm maize.

In mitochondria isolated from TMS cytoplasm, *B. maydis* (race T) toxin inhibited mitochondrial oxidation of a mixture of malate and pyruvate and stimulated the oxidation of succinate or exogenous reduced nicotinamide adenine di-

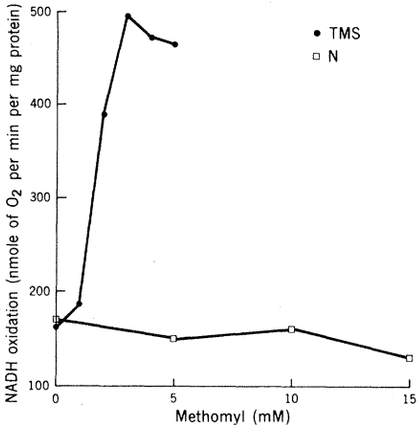

Fig. 1 (left). The effect of varying methomyl concentrations on the substrate state 4 rate of exogenous NADH oxidation by mitochondria isolated from etiolated TMS and N seedlings. Mitochondria were isolated from TMS and N cytoplasms of the single cross Wf9XB37 (backcrossed at least 15 times) (*13*), and mitochondrial protein was determined (*14*). Mitochondria (0.7 to 0.8 mg of protein) were added to a continuously stirred 4-ml reaction vessel (28° ± 0.2°C) containing 200 m*M* sucrose, 10 m*M* *N*-(tris-[hydroxy methyl] 2-amino) ethane sulfonic acid (*p*H 7.5), 1 m*M* $MgSO_4$, 10 m*M* KCl, 4m*M* KH_2PO_4, and 1 mg of BSA per milliliter. Exogenous NADH (2 μmole) was added to the mitochondrial suspension 2 minutes after the initial addition of mitochondria and the methomyl was added 1 minute later. Dissolved oxygen was measured polarographically by a Clark oxygen electrode fitted into the top of the reaction vessel and recorded on a dual-channel strip chart recorder along with percentage of transmittance measurements made with the reaction vessel placed in the light path of a modified Bausch & Lomb spectronic 70 spectrophotometer.

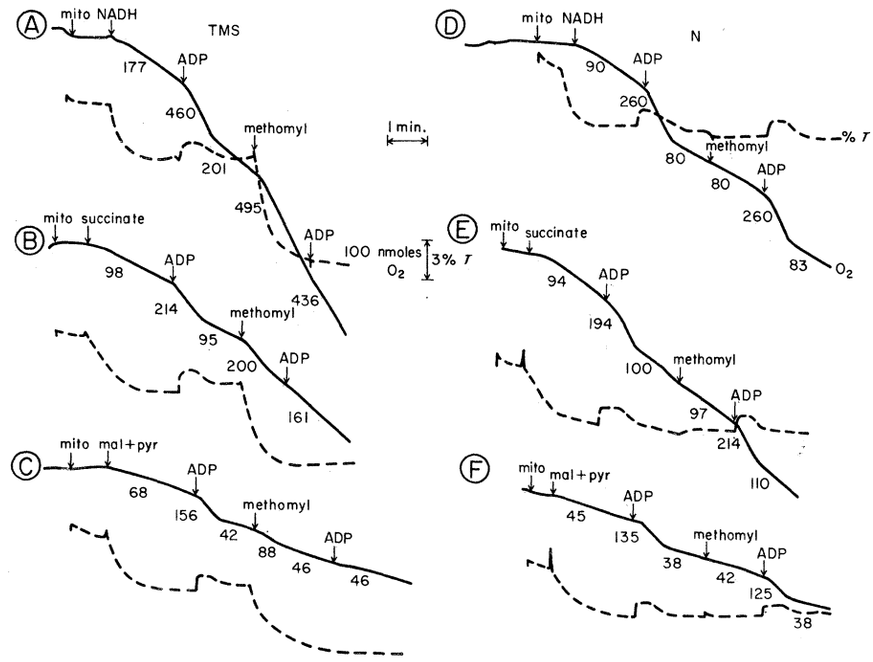

Fig. 2 (right). The effect of methomyl on various oxidative, phosphorylative, and transport parameters of isolated N and TMS mitochondrial suspensions. The numbers immediately adjacent to the solid lines (O_2 uptake) are the rates of O_2 uptake expressed as nanomoles of oxygen per minute per milligram of protein. The dashed line represents the percent of 520-nm light through the mitochondrial suspension, with a downward deflection representing an increase in percent *T*, or mitochondrial swelling. The experimental technique was the same as that given in the legend of Fig. 1. Amounts of added compounds as indicated were 40 μmole of succinate, 40 μmole of malate, 40 μmole of pyruvate, 2 μmole of NADH, 300 nmole of adenosine diphosphate, and methomyl to give a final concentration of 3 m*M*.

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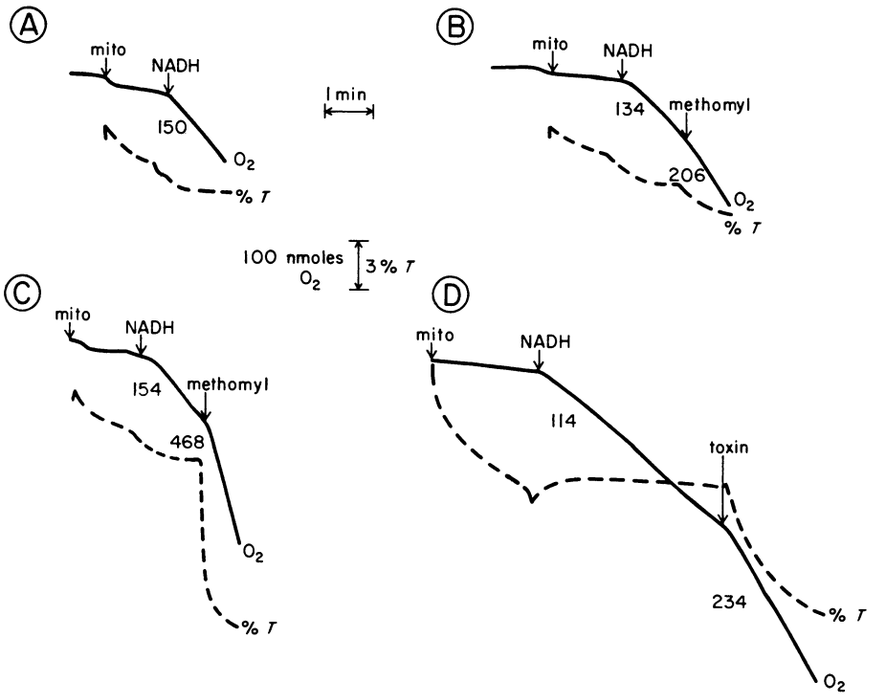

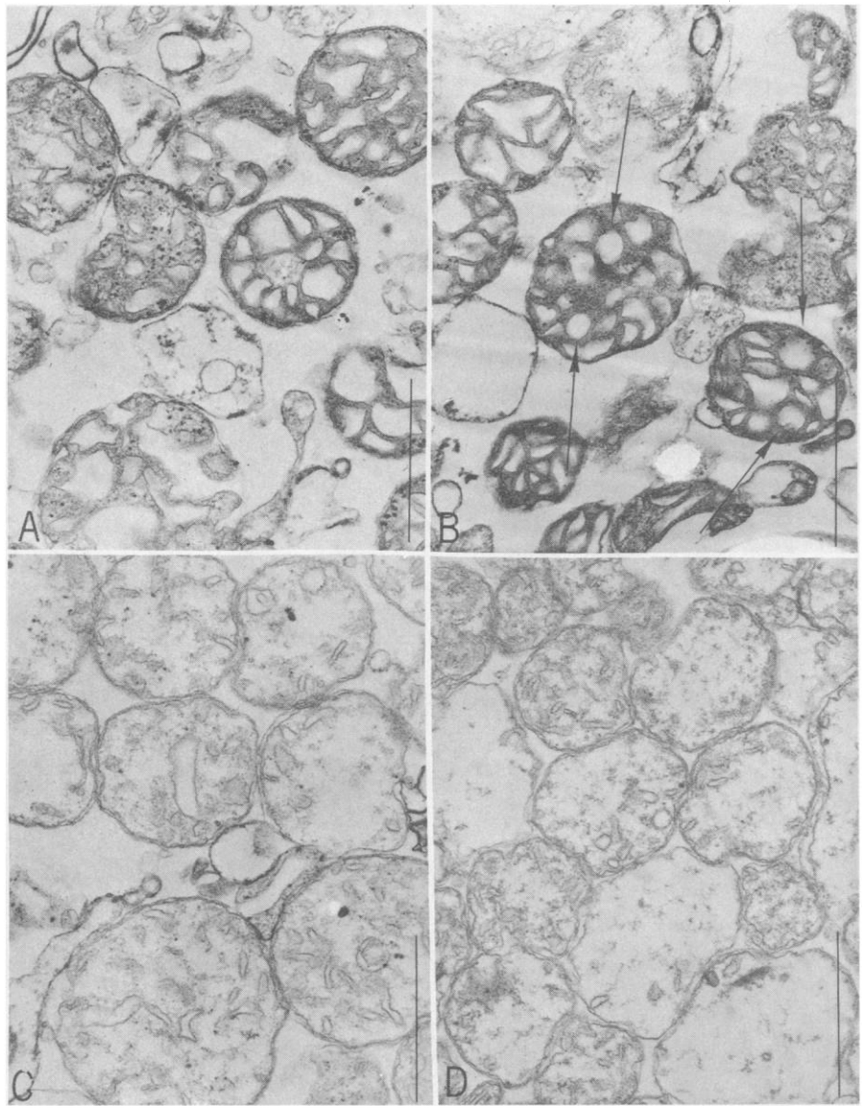

nucleotide (NADH) (*4*). These studies indicated a general correlation of the effects of toxin on phosphorylation, swelling, and spectrophotometrically observable membrane alterations. Given these clear differential effects of *B. maydis* (race T) toxin, we have studied the possible methomyl effects by looking at similar factors in mitochondria isolated from TMS and N etiolated seedlings.

In the first of these studies methomyl was added to isolated TMS mitochondria which were oxidizing exogenous NADH in substrate state 4. The rate of exogenous NADH oxidation after the addition of 3 m*M* methomyl increased by about 200 percent (Fig. 1). Mitochondria isolated from N cytoplasm seedlings showed only a slight reduction in the rate of exogenous NADH oxidation under comparable conditions after the addition of methomyl (final concentration, 15 m*M*). In other experiments both active and passive swelling were stimulated in TMS mitochondrial suspensions by methomyl, but were unaffected in N mitochondria by methomyl concentrations approximately five times those that had an optimal effect on TMS mitochondria. Methomyl effects are shown in Fig. 2, where simultaneous polarographic O_2 and percentage of transmittance curves indicate differences in N and TMS responses nearly identical to those reported for the effects of *B. maydis* toxin (*4*).

Fig. 3 (top). The effects of methomyl and *B. maydis* (race T) toxin on isolated TMS mitochondria oxidizing 2 μmole of exogenous NADH. Experimental conditions were the same as those for Fig. 1, except that in (D) the reaction media contained 200 m*M* KCl, 20 m*M* tris-HCl (*p*H 7.5), and BSA (1 mg/ml). Downward deflections represent an increased percent *T*. Concentrations of methomyl were 1 m*M* in (B) and 3 m*M* in (C). The effective concentration of *B. maydis* (race T) toxin (supplied by C. J. Arntzen) in (D) had no effect on isolated N mitochondria in comparable experiments. Fig. 4 (bottom). Electron micrographs of mitochondria isolated from TMS corn, showing the effects of 1 m*M* and 3 m*M* and 3 m*M* methomyl, and *B. maydis* (race T) toxin. Reaction conditions were the same as those for Fig. 1, except that the reaction media in (D) contained 200 m*M* KCl, 20 m*M* tris-HCl (*p*H 7.5), and BSA (1 mg/ml). Experimental conditions (O_2 uptake and percentage of transmittance) prior to fixation are the same as those given in Fig. 3. At the end of the Fig. 3 traces mitochondria were fixed in 2 percent glutaraldehyde in 100 m*M* phosphate buffer for 20 minutes. The preparations were postfixed in 2 percent OsO_4 in 100 m*M* phosphate buffer for 1 hour and dehydrated in a graded ethyl alcohol series; the tissue was embedded in Epon, with propylene oxide as a solvent. Specimens were poststained with lead acetate and observed on a Hitachi HU-11A electron microscope (line scales represent 1 μm).

These effects on isolated TMS (but not N) mitochondria include the stimulation of the rate of succinate or exogenous NADH oxidation, the stimulation of swelling, the inhibition of combined malate and pyruvate oxidation, and the inhibition of adenosine diphosphate (ADP)–induced state 3 oxidation.

Figure 3 shows the percentage of transmittance and O_2 curves of mitochondrial suspensions treated with methomyl or with *B. maydis* (race T) toxin before they were fixed for the electron micrographs presented in Fig. 4. The addition of 3 m*M* methomyl (Fig. 3C) or *B. maydis* (race T) toxin (Fig. 3D) resulted in an immediate and dramatic increase in the transmittance of light through the mitochondrial suspensions. This extensive swelling was not observed in the control (Fig. 3A) nor the 1 m*M* methomyl treatment (Fig. 3B). The electron micrographs in Fig. 4 confirm our interpretation of swelling, based on the percentage of transmittance curves in that mitochondria treated with 3 m*M* methomyl (Fig. 4C) or *B. maydis* (race T) toxin (Fig. 4D) exhibited a very dilute matrix and few cristae, yet had intact membranes. At 1 m*M* methomyl stimulated a moderate increase in the rate of NADH oxidation, but caused only a slight increase in the percentage of transmittance curve possibly due to a rounded cristae conformation (Fig. 4B).

While the observation of selective effects due to a potent insecticide on isolated maize mitochondria is significant in itself, the potential for the use of methomyl in assessing the difference in TMS and N maize mitochondria is likely of more interest to plant physiologists and geneticists. Studies aimed at delineating the precise site of *B. maydis* (race T) toxin binding to or its effect on isolated mitochondria have not been as exact as possible, largely because of the still poorly defined nature of the toxin or toxins. Methomyl is a relatively simple compound to which a radioactive label can be incorporated (*9*). The fact that membrane components are made labile with compounds such as digitonin (*10*), combined with methomyl binding studies, should distinguish the functional or structural difference between N and TMS mitochondria, provided that the methomyl and *B. maydis* (race T) toxin effects are as similar as we hypothesize them to be.

In view of the previous whole plant studies of Humaydan and Scott (*8*), our work defines at the organelle level the nature of the selective damage of methomyl to whole plants. That methomyl and certain other carbamate insecticides have been reported to damage other plant species in specific circumstances (*11*) opens the question of whether the genetic vulnerability of these plants to certain insecticides is conferred cytoplasmically through the mitochondrial genome.

The magnitude of the *B. maydis* (race T)–induced epiphytotic through much of the corn belt in 1970 (*12*) called renewed attention to the developing problem of a lack of genetic diversity in many of our crop species. It now seems clear that, while plant breeders and geneticists have developed heritable nuclear genomes of limited diversity, the much smaller and largely overlooked cytoplasmic genome probably contains even less diversity, not only among varieties of one crop but between many crops as well. Further studies with methomyl and closely related compounds known to effect plant damage should be of value in assessing the homogeneity, or lack of it, of a portion of the cytoplasmic genome associated with mitochondria.

In summary, the methomyl effect on TMS mitochondria of maize seems (i) to provide the potential for understanding a component of the extranuclear mitochondrial genome and (ii) to discriminate between detrimental and positive effects often present with the use of man-introduced environmental contaminants such as pesticides.

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α_1-Antitrypsin: The Presence of Excess Mannose in the Z Variant Isolated from Liver

Abstract. *The Z variant of α_1-antitrypsin was isolated by a new technique from the liver of a patient homozygous for the Z allele of the protease inhibitor locus. The material was homogeneous and antigenically competent but had no protease inhibiting capacity. An interesting correlation was found between the subcellular localization and the carbohydrate composition of the Z variant from liver. Carbohydrate analysis of this glycoprotein showed an absence of galactose and sialic acid, an appreciable decrease in* N*-acetylglucosamine, and an almost twofold increase in mannose residues. These data indicate a considerable slowdown in the processing of the oligosaccharides of liver Z variant. In spite of the absence of sialyl residues, the liver Z variant was microheterogeneous by analytical isoelectric focusing. The isoproteins of liver Z variant coincided with those of asialo M variant in the focusing field.*

In persons homozygous for the Z allele of the Pi (protease inhibitor) locus, the concentration of α_1-antitrypsin in the plasma is decreased to 10 to 20 percent of the normal value and a substantial amount of antitrypsin is retained in the liver (*1*). Heterozygous carriers of this allele have a correspondingly higher concentration of the protein in the serum, but they too show accumulation of this material in the liver. These findings strongly suggest an impairment either in the biosynthesis or the secretion of the Z variant. The fact that glycoproteins other than antitrypsin do not accumulate in the liver in this condition and the reported substitution of a glutamyl residue with lysine in the Z variant isolated from serum (*2*) suggest that an alteration of the Z variant, rather than alteration of the glycoprotein processing apparatus, is the primary cause of retention. We isolated and analyzed Z variant from the liver to find out how and at what particular stage the biosynthesis of this glycoprotein was affected.

Liver tissue was obtained at autopsy from a 50-year-old female, Pi ZZ patient.

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