Southern Corn Leaf Blight: Susceptible and Resistant Mitochondria

Abstract. *Mitochondria isolated from etiolated shoots of blight-susceptible and blight-resistant corn plants were subjected, in various respiratory states, to the pathotoxin released by* Helminthosporium maydis *(race T). The addition of the pathotoxin to susceptible mitochondria caused respiratory rate and oxidative phosphorylation changes. The addition of pathotoxin to susceptible mitochondria suspended in a potassium chloride reaction medium induced an immediate and irreversible swelling regardless of the respiratory state of the mitochondria. This membrane swelling correlates with the observed respiratory and coupling effects of the pathotoxin. In all instances, mitochondria isolated from blight-resistant corn failed to exhibit any of the above responses to the pathotoxin.*

In 1969 and 1970 corn inbreds and hybrids having the *cms-T* (Texas) type cytoplasm for male sterility displayed a dramatically increased susceptibility to the southern corn blight fungus *Helminthosporium maydis* Nisikado and Miyake. This was attributed to the prevalence and widespread distribution of a new pathogenic race of *H. maydis*, identified as race T (*1*). The development and spread of this disease was so rapid in 1970 from the southern to the northern regions of the United States that the effects of the blight in terms of reduced yields took on near epidemic proportions. In most instances plants having *cms-T* cytoplasm were more severely blighted than comparable plants having normal (nonsterile) cytoplasm. This difference in the susceptibility of corn thus appears to be associated with the cytoplasm and probably is hereditarily transferred in an extranuclear and extrachromosomal manner (*1*). Of the many cytoplasmic components investigated, both chloroplasts and mitochondria are now known to contain DNA (*2*), even though neither displays inheritance characteristics that are totally dissociated from the nucleus of the cell (*3*).

The selective effects of the toxin of *Helminthosporium victoriae* on membrane characteristics of susceptible oats have been observed for several years (*4–7*). Scheffer and Pringle (*4*) reported that this toxin, victorin, caused increased respiration in intact seedlings and roots of susceptible oats, but that the succinoxidase activity of mitochondria from these plants was unaffected. It was discovered later (*5*) that mitochondria isolated from susceptible seedlings treated with victorin did exhibit little respiratory control (RCR) and very low ratios of adenosine diphosphate (ADP) to oxygen. Both RCR and ADP : O ratios are good indices of mitochondrial integrity and thus indicate that victorin did influence the activities of mitochondria. However, Black and Wheeler (*6*) reported that when victorin was added directly to isolated mitochondria it produced no effect on the oxidative-phosphorylative capacity. These workers thus ascribed the effect of victorin only indirectly to mitochondrial activity and were not able to correlate the observed increase in respiration (*4*) with an effect on the mitochondria. A more general membrane effect of victorin was ob-

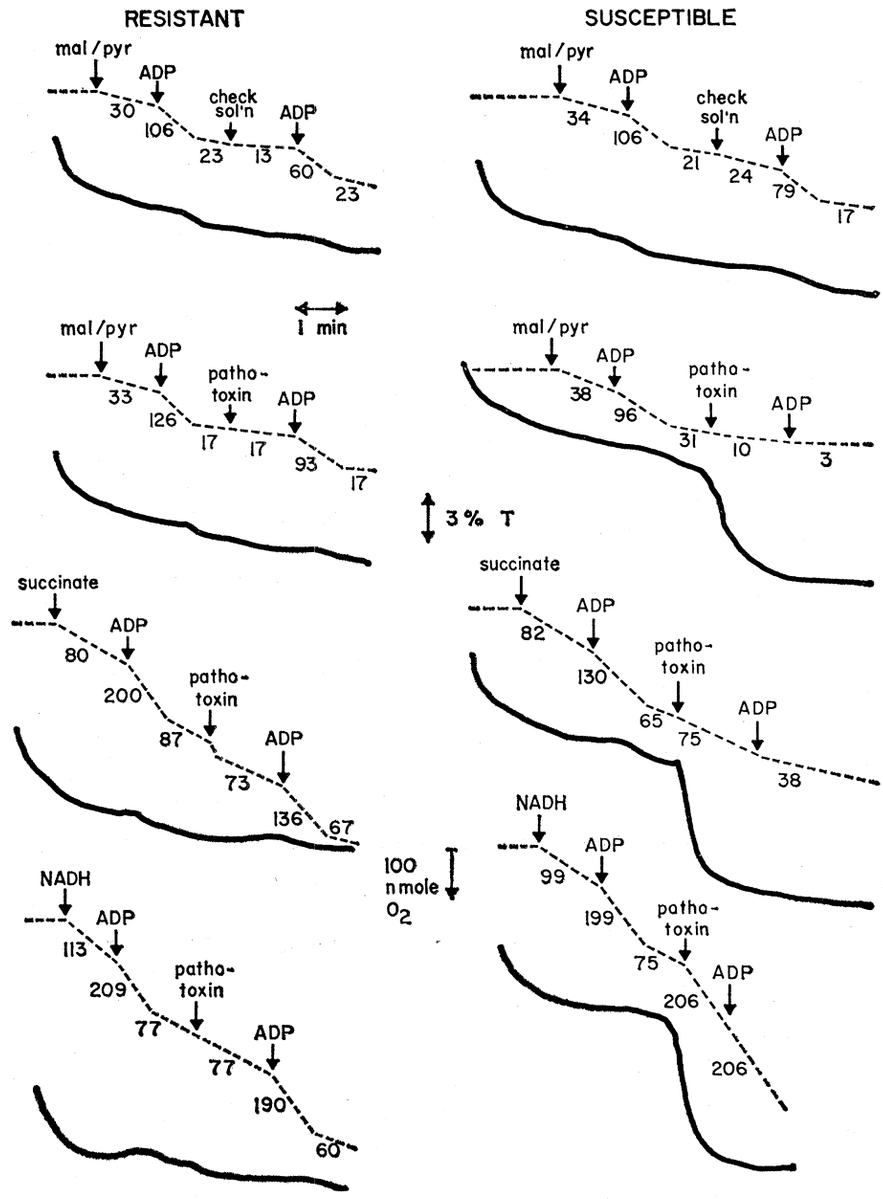

Fig. 1. The effect of *Helminthosporium maydis* race T pathotoxin on the swelling and coupling of mitochondria isolated from susceptible and resistant corn seedlings. Reaction conditions and substrate additions were as given for Table 1, but with the initial addition of 4 m*M* KH_2PO_4. The dashed line represents O_2 uptake and the solid line percentage of transmittance. Comparable amounts of pathotoxin (0.05 ml) were added to both susceptible and resistant mitochondria. Additions were: ADP, 300 nmole; NADH, 2 μmole; and succinate, 40 μmole. A downward deflection in the percentage of transmittance (*% T*) represents swelling.

served by Black and Wheeler (*6*) who reported that tissues treated with victorin lost much larger quantities of certain organic and inorganic (especially potassium) materials than control tissue did. Taking a similar tack, Samaddar and Scheffer (*7*) found that victorin stopped the uptake of exogenous amino acids and inorganic phosphate by susceptible, but not by resistant, tissue. On the basis of their data they hypothesized that the toxin produced a primary lesion in the plasma membrane and that it was possible that all other observed effects of the toxin were secondary to membrane damage.

A pathotoxin has been isolated but not characterized from cultures of *H. maydis* race T, the fungus responsible for the present infestation of southern corn blight (*1, 8*). This pathotoxin, in very small quantities, has been found (*1, 8*) to have an extremely deleterious effect on the root growth of susceptible, but not of resistant, corn seedlings. We have found that this corn blight pathotoxin also has a profound effect on mitochondria isolated from susceptible etiolated corn shoots (*cms-T* cytoplasm), but not on mitochondria isolated from resistant plants (normal cytoplasm). This is in contrast to the observed lack of effect of victorin on isolated oat mitochondria.

Mitochondria were isolated from 3-day-old etiolated corn shoots (*Zea mays* L.) as previously reported (*9*). Varieties of corn were: corn blight susceptible, Wf9 *cms-T* × M14; and corn blight resistant, PAG 15029. Final suspension of mitochondria was in 400 m*M* sucrose (*p*H 7.4). Experiments were performed with the addition of mitochondria (approximately 0.75 mg of protein) to a KCl reaction medium (details are given in Table 1 and Fig. 1 and 2).

In these experiments the following general effects of corn blight pathotoxin on mitochondria isolated from susceptible corn plants were observed: (i) respiration was either stimulated or inhibited depending on the substrate being oxidized, (ii) respiratory control and ADP : O ratios were reduced, (iii) mitochondria swelled at an accelerated rate in a medium of KCl under either passive or active conditions, and (iv) when treated with the toxin, mitochondria failed to contract after addition of substrate. In all instances comparable experiments were carried out with mitochondria isolated from resistant seedlings and none of the above effects of the pathotoxin were observed.

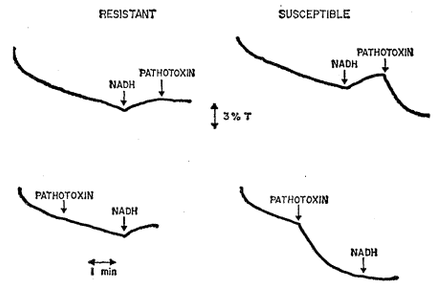

Fig. 2. The effect of *Helminthosporium maydis* race T pathotoxin on the swelling of mitochondria isolated from susceptible and resistant corn seedlings. Reaction conditions were as given for Table 1. One micromole of NADH and comparable amounts of pathotoxin (0.05 ml) were added as indicated. A downward deflection in the percentage of transmittance represents swelling with the reverse representing contraction.

The pathotoxin was produced as previously reported (*1*). Relative concentration curves of the effects of the pathotoxin were obtained by using mitochondria isolated from blight-susceptible shoots. Results are presented in Table 1 which show the effects of the pathotoxin in varying concentrations on both swelling of mitochondria and oxidation by mitochondria of malate-pyruvate. On the basis of these data, experiments were carried out, as reported in the remainder of this report, in which 0.05 ml of the pathotoxin solution was used. To test the resistance of the mitochondria isolated from resistant plants, up to 2 ml of the pathotoxin solution was present in the 4 ml of reaction medium. In all cases, under controlled conditions, this high concentration of pathotoxin was without effect.

Table 1. The effect of relative concentrations of pathotoxin on the swelling of isolated mitochondria and their oxidation of malate-pyruvate. Isolation and final suspension of mitochondria from etiolated corn shoots were as previously reported (*9*). Measurement of the rate of O_2 uptake was made with a Clark oxygen electrode in a 4-ml reaction solution (27° ± 0.2°C) containing 200 m*M* KCl, 20 m*M* tris-HCl (*p*H 7.4), and 1 mg of bovine serum albumin per milliliter. Light transmittance of the mitochondrial mixture was measured by fitting the reaction vessel in the light path of a modified Bausch & Lomb Spectronic 70 spectrophotometer. Both polarographically measured O_2 and percentage of transmittance were recorded simultaneously on a dual channel recorder. Oxidation was initiated by the addition of 40 μmole of malate and 40 μmole pyruvate. Protein was determined by the method of Lowry *et al.* (*11*). Change in transmittance is given as ΔT for 2 minutes after pathotoxin addition. The rate of O_2 uptake is expressed in nanomoles of O_2 per minute per milligram of protein.

Pathotoxin (ml)	Rate of O_2 uptake: Before pathotoxin	Rate of O_2 uptake: After pathotoxin	Pathotoxin-induced inhibition (%)	ΔT (%)
0	53	53	0	0.2
0.001	43	43	0	.6
.005	46	21	54	6.3
.01	43	11	75	7.4
.05	53	11	80	9.2
.10	50	18	64	10.1
.20	39	11	73	

When phosphate was not present in the reaction medium the rates of oxidation of malate-pyruvate or succinate by mitochondria from susceptible corn were reduced 75 and 17 percent, respectively, while the rate of oxidation of extramitochondrial NADH (reduced form of nicotinamide adenine dinucleotide) was increased 133 percent. Similar changes in the rates of oxidation of malate-pyruvate and extramitochondrial NADH were observed when the mitochondria were respiring under state 4 (no ADP present) conditions (Fig. 1). No inhibition of state 4 succinate oxidation was observed. After the addition of the pathotoxin, the subsequent addition of ADP did not stimulate respiration, rather it reduced the rates of oxidation of malate-pyruvate and of succinate and did not affect the oxidation rate of NADH (Fig. 1). This was unlike the addition of ADP to mitochondria (respiring in state 4) from resistant corn which caused a burst in the rate of respiration [state 3 (in the presence of ADP)] and then a falling back to the state 4 rate when all of the ADP had been used (Fig. 1). There was also a considerable difference in the rates of respiration after the addition of the pathotoxin to the mitochondria from susceptible corn depending on the substrate being oxidized (Fig. 1). The rate of oxidation of malate-pyruvate was reduced and that of succinate slightly increased by the pathotoxin, the rate of NADH oxidation was increased to the state 3 rate previously attained, and this rate was not increased further by the addition of ADP (Fig. 1). The lack of mitochondrial response to ADP after addition of pathotoxin is possibly due to some detrimental effect on the functioning of the mitochondria.

Figure 1 correlates the respiratory and coupling capacity of the mitochondria with their volume (percentage of transmission changes). A large increase in mitochondrial volume was observed when pathotoxin was added, but only

with mitochondria from blight-susceptible corn. Other compounds, such as gramicidin D and decenylsuccinic acid, cause similar volume changes along with an associated reduction in the coupling capacity of the mitochondria (*10*). Unpublished research from this laboratory shows that these are general mitochondrial effects and are not dependent on the mitochondria being from susceptible or resistant corn. It is probable that permeability changes are responsible for, or are correlated with, a reduction of coupling efficiency. The pathotoxin-induced rapid swelling of isolated mitochondria is independent of respiration (Fig. 2). Mitochondria swelled (KCl influx) after addition of pathotoxin under both passive and active conditions. This again corresponds to our previous experience with gramicidin D (*10*), with the exception that when pathotoxin-induced swelling was complete the addition of substrate would not cause a contraction as it did when the swelling was induced by gramicidin D.

Several important conclusions emerge from the data. First, the mitochondria isolated from blight-susceptible corn were adversely affected by pathotoxin from *H. maydis* race T, while no such effects were observed in mitochondria isolated from blight-resistant corn. Since the type of cytoplasm seems to be the determining factor in susceptibility or resistance of corn plants to race T of the southern corn blight fungus, mitochondria, with their inheritance capacity, could be a primary site of pathotoxin effect. The importance of mitochondrial respiration and production of adenosine triphosphate to the functioning of the whole plant is without question. The lack of functional mitochondria would spell quick death to diseased cells. Second, the effects of the pathotoxin on isolated mitochondria are in all instances concerned with membranes. Such a finding leaves the door open to comparable research involved with a myriad of biological reactions associated with the plasmalemma and membranes of other cytoplasmic organelles. In short, the research reported here is merely an introduction to a much more detailed study of the effects of the pathotoxin from *H. maydis* race T on cytoplasmic structure and function.

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Superior Colliculus: Some Receptive Field Properties of Bimodally Responsive Cells

Abstract. *Many cells in the intermediate and deep gray layers of the superior colliculus of the cat respond to both auditory and visual stimuli. These cells have similar receptive fields for both modalities and are directionally selective for both modalities, requiring stimuli moving laterally away from the animal. Perhaps cells that integrate auditory and visual information participate in the control of orienting and following responses to stimuli of both modalities.*

Several anatomical and physiological studies have suggested that the mammalian superior colliculus is primarily involved in the processing of visual information. The superficial gray and optic layers (superficial layers) of the cat superior colliculus receive both crossed and uncrossed projections from the optic nerve and an ipsilateral projection from the visual cortex (*1*). Cells in these layers have well-defined visual receptive fields (*2*).

The colliculus may also be involved in the processing of auditory and somatosensory information. The intermediate and deep gray layers (deeper layers) receive ascending projections from the inferior colliculus, spinal cord, and trigeminal nuclei (*3*). The deeper collicular layers also receive descending input from temporal cortex and postcentral gyrus (*4*). In the intact, unanesthetized cat, auditory and somatosensory stimuli drive a large number of cells in the deeper collicular layers (*5*). The stimuli required to drive these cells have not, however, been studied in detail.

I have found that if a cell responds to both auditory and visual stimuli, several of the receptive field properties are similar for both modalities. In particular, most cells responding to auditory and visual stimuli are directionally selective in response to both stimulus modalities, that is, they respond better to movement of the stimulus in one direction than to movement in the opposite direction. For both modalities, the horizontal component of the preferred direction was toward the periphery of the contralateral field.

An animal was prepared for recording several weeks before the actual experiment. Under Nembutal anesthesia, a well with a screw-on cap was implanted in the skull overlying the superior colliculus. At the same time, four bolts were cemented into the skull anterior to the plug. On the day of the experiment, the animal was anesthetized with halothane and nitrous oxide, intubated, and placed in a Horsely-Clarke stereotaxic apparatus facing a tangent screen. Supports were attached to the bolts, and the eye, ear, and mouth bars were removed. Thus, no pressure points or open wounds were present during recording; anesthesia could be discontinued. The animal was paralyzed with a mixture of gallamine and tubocurarine (*6*) and artificially respired. These experiments required the use of unanesthetized animals because previous experiments have shown that sensory stimuli do not drive cells in the deeper layers of the superior colliculus of anesthetized cats (*7*).

The cap was then removed from the well and a tungsten microelectrode (*8*) was lowered through the well to the superior colliculus. When the electrode isolated a single unit, a variety of visual, auditory, and somatosensory stim-