Comparative Effects of Herbicide Dicamba and Related Compound on Plant Mitochondrial Bioenergetics

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ABSTRACT: The herbicide dicamba (3,6-dichloro-2-methoxybenzoic acid) was evaluated for its effects on bioenergetic activities of potato tuber mitochondria to elucidate putative mechanisms of action and to compare its toxicity with 2-chlorobenzoic acid. Dicamba (4 μmol/mg mitochondrial protein) induces a limited stimulation of state 4 respiration of ca. 10%, and the above concentrations significantly inhibit respiration, whereas 2-chlorobenzoic acid maximally stimulates state 4 respiration (ca. 50%) at about 25 μmol/mg mitochondrial protein. As opposed to these limited effects on state 4 respiration, transmembrane electrical potential is strongly decreased by dicamba and 2-chlorobenzoic acid. Dicamba (25 μmol/mg mitochondrial protein) collapses, almost completely, $\Delta\psi$; similar concentrations of 2-chlorobenzoic acid promote $\Delta\psi$ drops of about 50%. Proton permeabilization partially contributes to $\Delta\psi$ collapse since swelling in K-acetate medium is stimulated, with dicamba promoting a stronger stimulation. The $\Delta\psi$ decrease induced by dicamba is not exclusively the result of a stimulation on the proton leak through the mitochondrial inner membrane, since there was no correspondence between the $\Delta\psi$ decrease and the change on the O_2 consumption on state 4 respiration; on the contrary, for concentrations above 8 μmol/mg mitochondrial protein a strong inhibition was observed. Both compounds inhibit the activity of respiratory complexes II and III but complex IV is not significantly affected. Complex I seems to be sensitive to these xenobiotics. In conclusion, dicamba is a stronger mitochondrial respiratory chain inhibitor and uncoupler as compared to 2-chlorobenzoic acid. Apparently, the differences in the lipophilicity are related to the different activities on mitochondrial bioenergetics.
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KEYWORDS: **Herbicide; Mitochondria; Solanum tuberosum; Aromatic Carboxylic Acids; Dicamba**

INTRODUCTION

Studies of the effects of herbicides and other xenobiotics on cellular metabolism are mandatory to understand the mechanisms of action. The mitochondrial fraction is a good model for the study of cell toxicity of xenobiotics in energy metabolism, since data from mitochondrial studies are reasonably correlated with cytotoxicity parameters reported for cell cultures and whole organisms [1–3]. These organelles provide the eukaryotic cell of nonphotosynthetic tissue with more than 90% of its total energy requirements. Suspensions of plant mitochondria can be obtained with a high degree of purity and integrity [4] and changes in activity can be studied by several methods: ion permeability, swelling, polarography, evaluation of transmembrane potentials and enzyme activities. Several xenobiotics interact with mitochondria, being classified, according to their effects, as uncoupling agents, electron-transport inhibitors, or energy-transfer inhibitors [5].

There are no studies of dicamba (3,6-dichloro-2-methoxybenzoic acid) effects on the mitochondria and the few published studies of the effects of dicamba were carried out on cells, plants, and animals and have only an agrochemical purpose [6–9].

Aquatic plant toxicity tests are frequently performed as environmental risk assessments to determine the potential impacts of contaminants on primary producers; tests performed in *Slenastrum* and *Lemna* do not show any sensitivity to dicamba [10].

Dicamba has been studied for its bioenergetics toxicity interactions in comparison to 2-chlorobenzoic acid, because of the similarities of their chemical structures (Figure 1). These studies are of obvious relevance to toxicology since most of the energetic and intermediary

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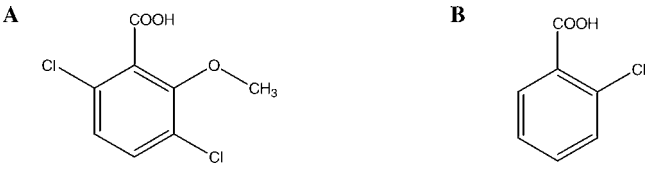

FIGURE 1. Structures of dicamba (A) and 2-chlorobenzoic acid (B).

metabolic compounds are generated at the expense of mitochondrial respiration. Therefore, interactions of xenobiotics with mitochondrial functions may severely affect the general metabolism.

EXPERIMENTAL METHODS

Preparation of Mitochondria

Potato tubers (*Solanum tuberosum*, L.) were obtained from the local market. Mitochondria were isolated and purified according to a procedure involving Percoll gradient centrifugation as a terminal purification step [4], except that 22% Percoll instead of 28% was used. The mitochondrial fraction was collected from the Percoll gradient with a Pasteur pipette and washed twice by centrifugation at 30,000 $\times$ g for 5 min in a washing medium containing mannitol (300 mM), bovine serum albumin (BSA; 0.1%), and Hepes (10 mM; pH 7.2). Protein was determined by the biuret method, using BSA as standard [11].

Measurement of Respiratory Activities

Oxygen consumption of isolated mitochondria was measured polarographically using a Clark-type oxygen electrode connected to a suitable recorder, in a closed water-jacketed 1-mL chamber with magnetic stirring, at 25°C [12]. Respiration rates were calculated assuming an oxygen concentration of 500 nAt O/mL in the experimental medium at 25°C. The standard respiratory medium consisted of 250 mM sucrose, 20 mM KCl, 5 mM $MgCl_2$, 5 mM K_2HPO_4, and 15 mM Hepes (pH 7.2). State 3 was elicited by adding 1.5 mM adenosine 5′-diphosphate (ADP), and uncoupled respiration by adding 1 μM carbonyl cyanide *p*-trifluoromethoxyphenyl hydrazone (FCCP); as respiratory substrates we used Malate/pyruvate, succinate, NADH, and ascorbate/TMPD.

The effects of xenobiotics on oxidative phosphorylation were estimated by determination of ADP/O ratios. Adenosine 5′-triphosphate (ATP) synthesis was assayed by a HPLC procedure according to the method described by Stocchi [13].

The Mitochondrial Transmembrane Potential

A tetraphenylphosphonium (TPP^+)-sensitive electrode, prepared according to Kamo et al. [14], was used to estimate the potential across the mitochondrial membrane. TPP^+ uptake was measured from the decreased TPP^+ concentration in the medium sensed by the electrode. The potential difference between the selective electrode and the reference (calomel electrode) was measured with an electrometer and recorded continuously in a Linear 1200 recorder. The voltage response of the TPP^+ electrode to log [TPP^+] was linear with a slope of 59 $\pm$ 1, at 25°C, equating well with the Nernst equation.

Mitochondria (0.5 mg protein) were resuspended in 1 mL of the standard respiratory medium (the same medium as described for the oxygen consumption experiments), at 25°C, with constant stirring. TPP^+ was added to a final concentration of 3 μM, i.e., an amount low enough to avoid any detectable toxic effects on mitochondria with the respiratory substrates. The TPP^+ uptake was initiated by the addition of substrate. The $\Delta\psi$ was estimated as indicated by Kamo et al. [14] from the equation

$$\Delta\psi(\text{mV}) = 59\log(v/V) - 59\log(10^{\Delta E/59} - 1)$$

where v and V are the volumes of mitochondrial and incubation medium, respectively, and ΔE is the deflection of the electrode potential from the baseline. The matrix volume of 1.1 μL/mg mitochondrial protein was considered. The above equation was derived assuming that TPP^+ distribution between the mitochondria and the medium follows the Nernst equation and that the law of mass conservation is applicable. No correction was made for the passive binding of TPP^+ to the mitochondrial membranes, since the purpose of the experiments was to show relative changes in potentials rather than absolute values. Calibration runs in the presence of dicamba excluded any direct interference of the herbicide in the electrode signal.

Enzymatic Activities

Succinate dehydrogenase activity was measured spectrophotometrically by the reduction of 2,6-dichlorophenolindophenol at 600 nm in the presence of phenazine methasulfate [15]. The reaction was performed in 1 mL of the standard reaction medium supplemented with 5 mM succinate, 2 μM rotenone, 0.1 μg antimycin A, 1 mM KCN, 0.025% Triton X-100 at 25°C, and 0.5 mg protein of disrupted mitochondria (two cycles of freezing and thawing).

Succinate cytochrome *c* reductase activity was measured spectrophotometrically [16] at 25°C by following

the reduction of oxidized cytochrome *c* by the increase in absorbance at 550 nm. The reaction was initiated by the addition of 5 mM succinate to 3 mL of the standard reaction medium supplemented with 2 μM rotenone, 1 mM KCN, 54 μM of cytochrome *c*, and 0.5 mg protein of broken mitochondria.

Cytochrome *c* oxidase activity was measured polarographically [17] at 25°C in 1 mL of the standard reaction medium supplemented with 5 mM succinate, 2 μM rotenone, 10 μM cytochrome *c*, and 0.5 mg protein broken mitochondria. The reaction was initiated by the addition of 5 mM ascorbate plus 0.25 mM TMPD.

Mitochondrial Swelling

Mitochondrial osmotic swelling [18] was monitored by detecting turbidity, at 520 nm, on a Spectronic Genesys 2PC spectrophotometer, in a thermostatic chamber with magnetic stirring at 25°C. Media were 44% isosmolar (100% = 270 isosmolar), containing 54 mM K-acetate, 10 mM Hepes, 0.1 mM Tris-EGTA, and 0.2 mM Tris-EDTA (pH 7.1). The cuvette contained 2.0 mL of the reaction medium plus 0.3 mg protein, 200 μM propranolol, 10 μM atractyloside, and 1 μM antimycin A.

Chemicals

All reagents were of analytical grade for research. Dicamba was purchased from Riedel-Häen; 2-chlorobenzoic acid and phenylacetic acid were from Merck and the other compounds were from Sigma.

Treatment of the Data

Results are presented with the values of ±SEM from at least three independent experiments. Some figures are records of individual experiments representative of three or more replicates.

RESULTS

Xenobiotic Effects on the Mitochondrial Respiration

The effectiveness of a fixed amount of dicamba and 2-chlorobenzoic acid (Figure 1) decreased with increasing mitochondrial protein concentration when membrane potentials and rates of oxygen consumption were assayed (results not shown). For this reason the assays were always performed with a similar mitochondrial concentration.

The effects of dicamba and 2-chlorobenzoic acid on plant mitochondria respiration are displayed in Figure 2, using succinate as the respiratory substrate; the data was obtained for state 4 and state 3. Dicamba at 4 μmol/mg mitochondrial protein promotes a small stimulation on state 4 respiration, of ca. 10% of the control value, but higher concentrations significantly inhibit state 4 respiration, while 2-chlorobenzoic acid stimulates state 4 respiration as a function of concentration.

For state 3 succinate-supported respiration both compounds behave as inhibitors, dicamba being the most effective. Dicamba (25 μmol/mg mitochondrial protein) promotes a decrease in state 3 of about 85%, whereas 2-chlorobenzoic acid at the same concentration inhibits by 50% of the control. The observed effect of dicamba on state 4 respiration suggests that dicamba does not have any uncoupling effect.

As a control we performed the assays described in Figure 2 with a modification. First we put the mitochondria in a medium preimplemented with substrate, and 5 min later dicamba or 2-chlorobenzoic acid was added. The purpose of this control was to see if the mitochondria preincubated with dicamba prior to substrate addition provoked irreversible damage on the respiratory complexes, with a limitation in the maximal respiratory rates. From the obtained results we saw that the preincubation with dicamba before the substrate addition caused no irreversible damage on the respiratory complexes, since after 5–10 min of the dicamba addition the

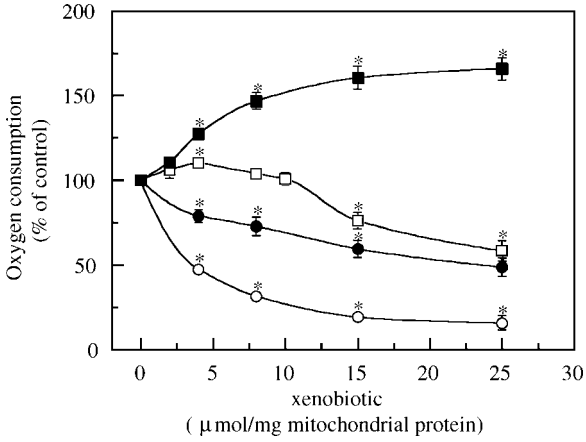

FIGURE 2. Effects of dicamba (open symbol) and 2-chlorobenzoic acid (filled symbol) on respiratory rates of mitochondria potato tuber. Mitochondria (0.3 mg) were incubated in 1 mL of the respiratory standard medium, at 25°C, in the presence of used xenobiotic concentrations (0, 2, 4, 8, 15, 25 μmol/mg mitochondrial protein), for 5 min. State 4 respiration (□, ■) was initiated by the addition of 10 mM succinate. State 3 respiration (○, ●) energized by 10 mM succinate was initiated by the addition of 1.5 mM ADP. ADP was added 2 min after the initiation of state 4 respiration. Control values for succinate-supported respirations are expressed in nmol O_2/(mg^{-1} protein min). State 4, 49.2 (±12.2); state 3, 186 (±65.4). Values are the means ± SEM of six independent experiments. *Values statistically different from control ($p < 0.05$).

effects were observed (data not shown) and the maximal effects were identical to the results obtained when a preincubation with dicamba was performed after the substrate addition.

Oxygen consumption of controls in the absence or presence of any of the used xenobiotics was completely inhibited by the addition of antimycin A (not shown), indicating that the oxygen consumption was exclusively from the respiratory activity.

Xenobiotic Effects on the Mitochondrial Transmembrane Potential

To better evaluate the action of the tested compounds on the redox activities and phosphorylation, the alterations of the transmembrane potential ($\Delta\psi$) developed by mitochondria at the expense of substrate oxidation were followed, as previously described (Figure 3).

After the addition of succinate, mitochondria developed a potential of about −213 mV and the xenobiotics promoted a $\Delta\psi$ dissipation, as a function of added concentrations.

Dicamba is the most effective agent in promoting $\Delta\psi$ dissipation, this dissipation already being apparent in the presence of 2 μmol/mg mitochondrial protein. Dicamba, at 25 mM, collapses $\Delta\psi$ almost completely, whereas a similar concentration of 2-chlorobenzoic acid only depresses $\Delta\psi$ by about 50% (Figures 3A and 3B). Dissipation of $\Delta\psi$ is not a consequence of a progressive stimulation of state 4 respiration, as could be expected if $\Delta\psi$ collapse were only the result of a membrane permeabilization to protons.

Xenobiotic Effects on the Oxidation of Different Substrates

The oxidation of different substrates for FCCP-stimulated respiration in the presence of dicamba and 2-chlorobenzoic acid (15 μmol/mg mitochondrial protein) were inhibited for most of the used substrates, the exception being ascorbate/TMPD where no significant inhibition was observed (Figure 4).

In mitochondria respiring malate/pyruvate (Figure 4), dicamba at 15 μmol/mg mitochondrial protein depresses the redox chain activity by about 54%, whereas 2-chlorobenzoic acid decreases the activity by 37%. With succinate (Figure 4), the inhibition observed for dicamba and for 2-chlorobenzoic acid at the maximum concentration used (15 μmol/mg mitochondrial protein) were 65 and 44% respectively. When NADH or ascorbate/TMPD was used as substrate (Figure 4), no significant differences were observed between dicamba and 2-chlorobenzoic acid. Furthermore, respiration supported by exogenous NADH was inhibited by about 30%, but no significant inhibition was observed for respiration promoted by ascorbate/TMPD, which is linked to cytochrome *c* oxidase.

Xenobiotic Effects on the Oxidative Phosphorylation

The influence of dicamba and 2-chlorobenzoic acid on phosphorylation was tested using succinate as the respiratory substrate (Figure 5). In the absence of any xenobiotic we obtained an ADP/O of about 1.8, but when dicamba or 2-chlorobenzoic acid was added at

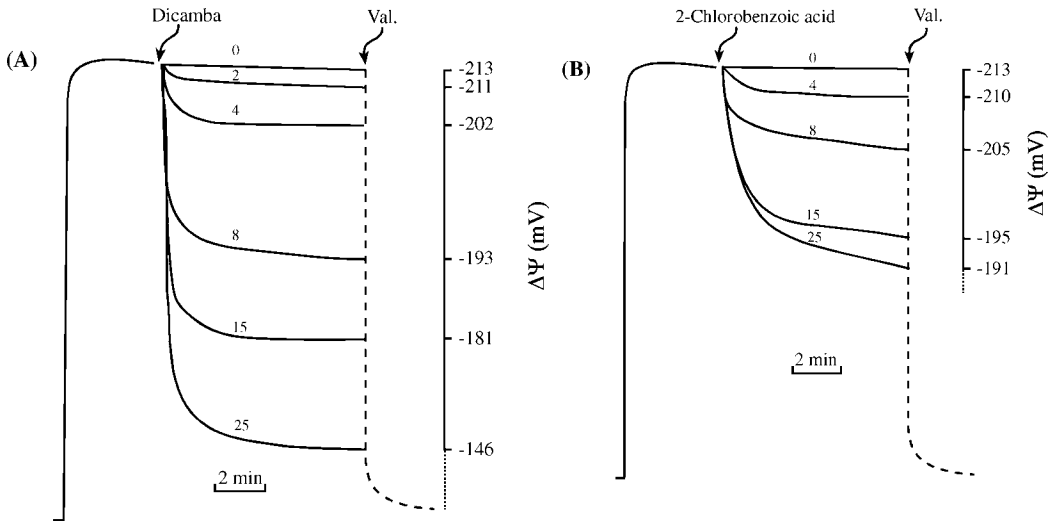

FIGURE 3. Effects of dicamba (A) and 2-chlorobenzoic acid (B) on transmembrane potential ($\Delta\psi$) supported by succinate. Mitochondria (0.3 mg) were added to the standard respiratory medium supplemented with 3 μM TPP^+. Xenobiotics were added at the indicated concentrations (0, 2, 4, 8, 15, 25 μmol/mg mitochondrial protein). Valinomycin was added at the end of each assay to elicit complete collapse of membrane potential. The traces represent typical recordings from several experiments with different mitochondrial preparations.

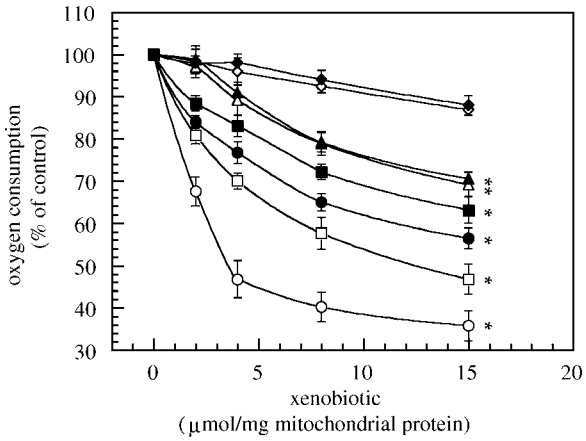

FIGURE 4. Effects of dicamba (open symbol) and 2-chlorobenzoic acid (filled symbol) on FCCP-uncoupled respiration for malate (□, ■), succinate (○, ■), exogenous NADH (△, ▲), and ascorbate/TMPD-supported respiration (◇, ♦) in potato tuber mitochondria. Assay media: malate (20 mM malate, 4 mM pyruvate, 1 mM TPP, and 1 mM NAD^+); succinate (10 mM succinate); exogenous NADH (1 mM NADH); ascorbate/TMPD (10 mM ascorbate and 0.75 mM TMPD). Control values are expressed in nmol O_2/(mg protein min): malate, 120 (±11.0); succinate, 198 (±30.1); exogenous NADH, 158 (±24.3); ascorbate/TMPD, 298 (±38.0). Values are the means ± SEM of three independent experiments.

2 μmol/mg mitochondrial protein the ADP/O decreased to 0.8 and to 1.2, respectively. For 15 μmol/mg mitochondrial protein the inhibition observed on the ATP synthesis were 74 and 40%, respectively for dicamba and for 2-chlorobenzoic acid. The sharp depression induced by 2-chlorobenzoic acid on the ADP/O was not accompanied by a sharp depression of ATP synthesis, since more than 50% synthesis was still observed at 15 μmol/mg mitochondrial protein, while ADP/O was nonmeasurable at this concentration. ADP/O was already nonmeasurable for dicamba at 8 μmol/mg mitochondrial protein, but ATP synthesis was still measurable at 15 μmol/mg mitochondrial protein.

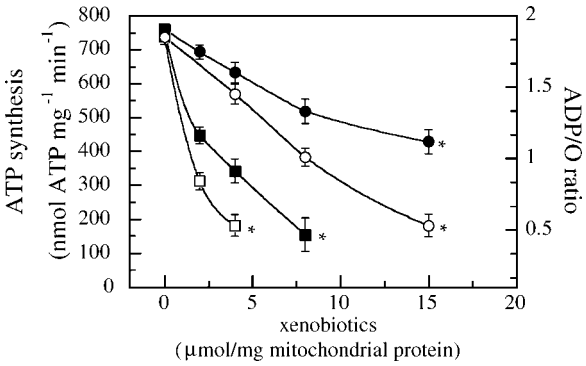

FIGURE 5. Effects of dicamba (open symbol) and 2-chlorobenzoic acid (filled symbol) on the oxidative phosphorylation of potato tuber mitochondria supported by succinate. Synthesis of ATP (●, ○), ADP/O ratio (■, □). Mitochondria (1 mg) were incubated in 2 mL reaction mediums (described in the Methods Section), at 25°C. Control values for ATP synthesis 760 (±82.0) nmol ATP/(mg mitochondrial protein min); ADP/O 1.85 (±0.053). Assays were performed in the conditions described in the Methods Section. Values are the means ± SEM of three independent experiments. * Values statistically different from control ($p < 0.05$).

Xenobiotic Effects on the Respiratory Complexes (II, III and IV) Activity

Studies of enzymatic activities of respiratory complexes II, III, and IV localized the components of the mitochondrial respiratory chain affected by dicamba and 2-chlorobenzoic acid (Figure 6). The effect of xenobiotics on the redox activities helps us to understand and explain not only the interaction with redox complexes but also the changes in the membrane potential. Dicamba (25 μmol/mg mitochondrial protein) markedly inhibits the succinate cytochrome *c* reductase activity by about 50% as well as succinate dehydrogenase activity by about 35%. The inhibition induced by 2-chlorobenzoic acid, at the same concentration, was only about 30 and 20%, respectively, for succinate cytochrome *c* reductase and succinate dehydrogenase. The terminal segment of the respiratory chain, cytochrome *c* oxidase, was not significantly inhibited by either xenobiotic.

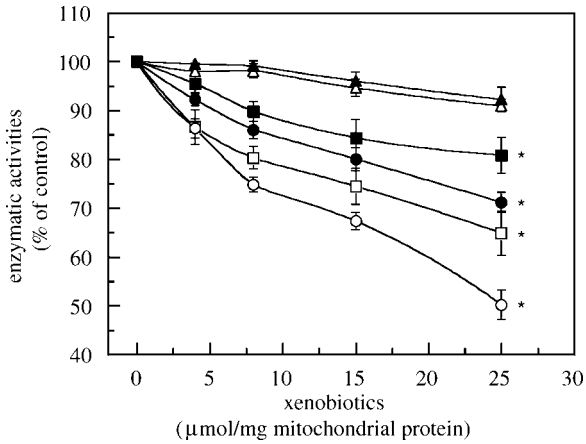

FIGURE 6. Effects of dicamba (open symbol) and 2-chlorobenzoic acid (filled symbol) on the respiratory complexes: succinate dehydrogenase (■, □), succinate cytochrome *c* reductase (●, ○), cytochrome *c* oxidase (▲, △). Mitochondria (1 mg) were incubated in 2 mL reaction mediums (described in the Methods Section), at 25°C. Assays were performed in the conditions described in the Methods Section. Control values: succinate dehydrogenase, 230 (±12.2) nmol O_2/(mg mitochondrial protein min); succinate cytochrome *c* reductase, 1.8 (±0.042) nmol cytochrome *c* reduced/(mg mitochondrial protein min); cytochrome *c* oxidase, 435 (±42.3) nmol O_2/(mg mitochondrial protein min). Values are the means ± SEM of three independent experiments.

Proton-Dependent Mitochondrial Swelling

Mitochondrial swelling was followed in potassium acetate (54 mM) isosmolar medium in the presence of different xenobiotics (Figure 7). The maximal valinomycin-dependent swelling stimulation was observed upon addition of FCCP (1 μM). Mitochondrial swelling occurs if a protonophore is present to promote the passage of protons from the matrix to the extramitochondrial reaction medium, permitting further acetate and K^+ influx [19]. Therefore, swelling promoted by valinomycin under the action of added xenobiotics is a consequence of protonophoric actions.

Both the tested xenobiotics can increase the permeability of inner mitochondria membrane to protons. Dicamba (Figure 7A) was the most efficient in inducing swelling as a function of concentration; 2-chlorobenzoic acid (Figure 7B) also induces swelling but in a smaller degree when compared with dicamba.

DISCUSSION

Mitochondrial transmembrane potential studies are of major importance in relating the effect of xenobiotics to the mitochondrial oxidative phosphorylation, since $\Delta\psi$ represents the main component of the electrochemical gradient and accounts for more than 90% of the total available energy [19] on nonphotosynthetic tissues.

From the strong $\Delta\psi$ dissipation promoted by the dicamba (Figure 3A) a strong stimulation should be expected on the state 4 respiration, since with the $\Delta\psi$ dissipation the imposed restriction by the proton motive force can be raised, but the obtained result on the state 4 O_2 consumption did not agree with a strong $\Delta\psi$ dissipation promoted by an increase on the proton leak. Even for 2-chlorobenzoic acid the stimulation observed in the state 4 respiratory rate is much lower than could be expected from the observed $\Delta\psi$ dissipation [20]. Therefore, we believe that the greater part of the $\Delta\psi$ dissipation must be as a result of an inhibitory effect on the redox chain complexes.

The results obtained on the state 3 (Figure 2) and on the uncoupled respiration (Figure 4) clearly indicate an inhibitory effect on some of the respiratory complexes.

The respiration rate was differentially inhibited by the two xenobiotics, depending on the nature of the respiratory substrate used. The exception was observed with the mitochondria respiring ascorbate/TMPD and exogenous NADH, since no significant difference was observed between dicamba and 2-chlorobenzoic acid (Figure 4). The results in Figure 4 points to the conclusion that complexes I, II, and III can be strongly inhibited, while the terminal segment of the respiratory chain (cytochrome *c* oxidase) cannot be significantly affected by these two xenobiotics. These results are further supported by the results obtained for succinate dehydrogenase, succinate cytochrome *c* reductase, and cytochrome *c* oxidase (Figure 6). Inhibition of succinate-supported respiration by dicamba and 2-chlorobenzoic acid (15 μmol/mg mitochondrial protein) was 55 and 35%, respectively, while at this concentration the inhibition of the respiration supported by exogenous NADH was about 30% for both xenobiotics. Considering that the oxidation of both substrates, succinate, pyruvate/malate, and exogenous NADH have a common electron-transfer pathway, it is tempting to conclude that the two xenobiotics, especially dicamba, may act on substrate transport [21], since the oxidation of the exogenous NADH was less inhibited than the oxidation of succinate or malate, and only NADH does not need to cross the inner mitochondrial membrane to be oxidized. Furthermore, the difference in the inhibition (%) observed between Figure 4 (oxygen consumption on FCCP-uncoupled respiration) and Figure 6

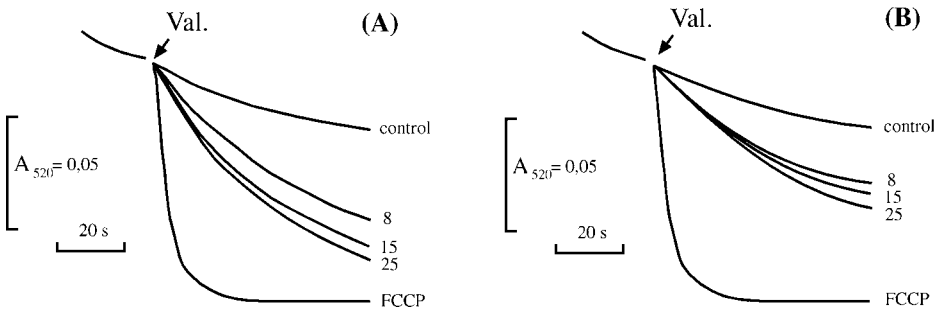

FIGURE 7. Effects of dicamba (A) and 2-chlorobenzoic acid (B) on the mitochondrial swelling. Swelling in 44% isosmolar potassium acetate (pH 7.1) supplemented with antimycin (1 μM), atractyloside (10 μM) and propranolol (200 μM), with BSA (0.1%). Xenobiotics were added as indicated in the figure (8, 15, 25 μmol/mg mitochondrial protein). Control represents the assay without any xenobiotic or FCCP. The line indicated by FCCP represents an experiment in which 1 μM FCCP was added; valinomycin (1 μM) was added in all the experiments like it is indicated. The traces are representative of a group of at least three independent experiments.

(respiratory complexes activities) is certainly the result of an inhibitory effect on the substrate transport across the membrane, since the enzymatic activities are obtained with disrupted mitochondria and for this reason membrane transport is not necessary, whereas FCCP-uncoupled respiration was obtained with intact mitochondria. We did not evaluate the activity of the exogenous NADH dehydrogenase, since in plant mitochondria three different NADH dehydrogenases are present [22].

Both xenobiotics can stimulate a proton leak through the mitochondrial inner membrane as was demonstrated by the swelling assays (Figure 7).

Independent of actions on the different complexes of the redox chain, we can also see from Figure 5 that dicamba and 2-chlorobenzoic acid can both interact with the phosphorylation system, by a similar mechanism to that already demonstrated for other xenobiotics [23,24]. The effect on the phosphorylation system is probably due to two different mechanisms; first, a direct effect on the F_0–F_1 ATPase, since ATPase activity was also inhibited (data not show), and second, by an inhibition on the nucleotides carrier and inorganic phosphate carrier, since the inhibition observed on the ATP synthase activity (Figure 5) was much greater than that obtained for ATPase.

In our laboratory we have tested the effect of a number of xenobiotics, including dicamba, on cell growth of nongreen potato tuber callus, and the results show that dicamba did not stimulate superoxide dismutase, catalase, or glutathione reductase (data not shown). Therefore, on the basis of these results we can assume that dicamba and 2-chlorobenzoic acid metabolisms do not produce any significant reactive oxygen species. Moreover, oxygen consumption supported by succinate of controls, in the absence or presence of any of the used xenobiotics, was completely inhibited by the addition of antimycin A (not shown), indicating that the oxygen consumption came exclusively from the respiratory activity.

If we compare the sensitivity of mitochondria to dicamba or to 2-chlorobenzoic acid with the sensitivity obtained with other compounds, like bromosulfophtalein [24], dinoseb [25], styrylchromone [26], and with many others, we can see that mitochondrial bioenergetics are much less affected by dicamba and 2-chlorobenzoic acid (a difference in the order of 10^3). This difference in the sensitivity is due to the lower lipophilicity of dicamba and 2-chlorobenzoic acid, and therefore, their incorporation in membrane lipid domains is much poorer than other xenobiotics. At higher concentrations like those used in our experiments, some dicamba can certainly incorporate into the membrane, the aromatic portion being immersed into the hydrophobic part, whereas the polar portion of the molecule remains in the hydrophilic surface. In fact, we tried to measure the partition of dicamba and 2-chlorobenzoic acid into submitochondrial particles, using a spectroscopic method described by Videira et al. [27], but it was impossible to measure because the level of incorporation was very small.

The differences observed in the effects of dicamba and 2-chlorobenzoic acid should be related to the differences in the chemical structure itself, which result in different lipophilicity. As a consequence those xenobiotics should present different extensions of mitochondrial membrane incorporation. Therefore, these differences should result from a different extension of a direct interaction with functional membrane proteins and/or boundary lipids, as it has been known for a long time that the functionality of many membranes proteins are strongly associated with the surrounding lipids [28].

In conclusion, the inhibitory action of dicamba and 2-chlorobenzoic acid on the phosphorylation efficiency of mitochondria could be, in the last analysis, due to an incorporation of dicamba into the mitochondrial membranes, causing an alteration on the surface charge density and a disturbance in the physicochemical and structural properties of the inner membrane, resulting in an unequivocal disturbance in the electron delivery between redox complexes (in particular between II and III) in the phosphorilative system, and, in addition, an increase of the permeability to the protons.

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