Interactions of various agrochemicals with cytochrome P-450-dependent monooxygenases of wheat cells

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Abstract

Levels of cytochrome P-450s and rates of monooxygenase activities were studied in microsomes prepared from wheat cell suspension cultures. Cytochrome contents and enzymatic activities, namely, the enzymatic systems responsible for chlorotoluron ring-methyl hydroxylation and N- demethylation, !auric acid hydroxylase, and cinnamic acid 4-hydroxylase , were enhanced after pretreatment of cells with 2,4-D, procloraz, mecoprop , chlorotoluron , and oxime ether safeners. Effects of various agrochemicals were also determined on chlorotoluron ring-methyl hydroxylase and N-demethylase, foJJowing their direct addition to microsomal preparations. Plant growth reg-ulators and fungicides, as well as piperonyl butoxide decreased both activities, tetcyclacis, and procloraz being markedly inhibitory. Naphthalic anhydride, oxime ether safeners, dichlormid, and tridiphane had only weak effects. The substrate specificities of chlorotoluron ring-methyl hydroxylase and N-demethylase were

also investigated using structural analogues of the herbicide. Diuron was the strongest inhibitor among the tested phenylureas. Other herbicides that can be metabolized by wheat affected both activities to different extents. However, diclofop enhanced only chlorotoluron N-demethylase.

Introduction

Higher plants are frequently able to metabolize the xenobiotics that they absorb. Among the enzymes responsible for the formation of primary metabolites, those which are involved in oxidative transformations, i.e., monooxygenases, are poorly characterized. In this study, we were interested in the enzymes responsible for the oxidative metabolism of the phenylurea herbicide chlorotoluron in wheat.

It has been shown that chlorotoluron is metabolized in wheat plants by ring-methyl hydroxylation and N-demethylation, followed by sugar conjugation (1), in agreement with previous studies (2). Two inhibitors of cytochrome P-450 monooxygenases, aminobenzotriazole (ABT) and piperonyl butoxide (PBO), enhanced the phytotoxicity of chlorotoluron in wheat (3). It has been demonstrated that the effect of ABT is due to an inhibition of chlorotoluron oxidative metabolism (4).

Further studies have shown that cultured wheat cells metabolize chlorotoluron by the same pathways as intact plants (5). In wheat cells, oxidative metabolism of chlorotoluron was inhibited by monooxygenase inhibitors such as ABT, tetcyclacis, and paclobutrazol, and enhanced by pretreat- ments with cyometrinil, 2,4-dichlorophenoxyacetic acid (2,4-D), and dichlormid (5). Recent results suggested that ring-methyl hydroxylation and N-demethylation of chlorotoluron in wheat cells

pretreated with cyometrinil could be catalyzed by different cytochrome P-450 monooxygenases (6).

In this paper, we have examined the effects of various agrochemicals on the *in vitro* metabolism of chlorotoluron by wheat cell microsomal preparations.

In a first series of experiments, cultured cells were pretreated with various pesticides or safeners. The capacities of the microsomal fractions to metabolize chlorotoluron were then compared.

Second, we have examined the direct effects of various agrochemicals on the *in vitro* metabolism of chlorotoluron. Some of these compounds have been chosen be- cause they inhibit cytochrome P-450 enzymes, as plants growth regulators (7-12), sterol biosynthesis inhibitors (9-12), or insecticide synergists (13). Herbicide safeners and synergists have also been included in this survey.

And finally, the substrate specificities of the monooxygenases which metabolize chlorotoluron have been evaluated by sup-plementing the assay mixtures with structural analogues of the herbicide or other herbicides metabolized by wheat plants or cell cultures (14-18).

Materials and methods

Chemicals. Labeled chlorotoluron (3- [3-chloro-p-tolyl]-l, 1-dimethylurea) was synthesized according to (6). Labeled lauric and cinnamic acids were from CEA (Gif/Yvette, France).

Laurie acid, cinnamic acid, and 2,4-D were purchased from Sigma. Procloraz was obtained from FBC Limited (Cambridge, UK), and oxime ether safeners (cyometrinil, (Z)-a-

[(cyanomethoxy)imino]benzene- acetonitile; oxabetrinil, a-[(l,3-dioxolan-2-ylmethoxy)imino]benzeneacetonitrile; CGA 133205, O-[1,3-dioxolan-2-ylmethyl]-2,2,2-trifluoro-4'-chloroacetophenone oxime) were from Ciba-Geigy. Tetcyclacis (5-(4-chlorophenyl]-3,4,5,9,10-pentaazatetra-cyclo[5.4.1.$0^{2,6}$.$0^{8,11}$]dodeca-3,9-diene) and fenpropimorph were obtained from BASF, and triadimenol and triadimefon from Bayer. Paclobutrazol came from ICI, naphthalic anhydride from Aldrich, and tridiphane from Dow Chemicals. Dr. R. Varsano (Hebrew University of Jerusalem, Rehovot, lsrae]) and Dr. I. Barta (Hungarian Academy of Sciences, Budapest, Hungary) provided PBO and dichlormid, respectively. Isoproturon, fenuron, and chlorbromuron were obtained from Rhone Poulenc Agrochimie, Chemical Service (Westchester, U.S.A.), and Cluzeau Info Labo (Ste Foy la Grande, France), respectively. Other phenylureas, as well as mecoprop, were purchased from Pestanal (Hannover, RFA). Chlorsulfuron was given by Rhone Poulenc Agrochimie. Atrazine and terbutryne were from Chemical Service. Diclofop was obtained from Hoechst.

Cell cultures and treatments. Achlorophyllous wheat cells *(Triticum aestivum* L., var. "Koga II") were routinely cultivated in Gamborg B5 medium with 2 mg/liter (9 µM) 2,4-D as previously described (5). Eight-day-old suspensions were subcultured at an inoculum density of 4 mg dry weight/ml. After 4 days of growth, the cells were put in fresh culture medium containing DMSO (controls) or various effectors dissolved in DMSO. Final solvent concentrations in culture media were less than 0.1% (v/v), and did not affect cell growth nor rates of oxygenase activities. Microsomal fractions were isolated immediately after subculturing, and 24, 48, or 72 hr later.

Preparation of microsomes. All experiments were carried out at 4°C.

The cultures were kept for I hr at 4°C, and then filtered through a stainless steel filter. Cells (55-65 g fresh weight) were rinsed with 50 ml of 0.l M potassium phosphate buffer (pH 7.5) and ground in a mortar for I min in 50 ml of grinding buffer containing 0.1 M phosphate (pH 7.5), 20% (w/v) glycerol, 20 mM ascorbic acid, 14 mM mercaptoethanol, I mM EDTA, I mM phenylmethylsulfonyl fluoride, and 10% (w/w) XAD-4 resin. After filtering and centrifuging the crude extract as previously described (6), the supernatant was centrifuged at 150,000g (Beckman 45Ti) for 45 min. The pellets were then resuspended in 50 ml of 0.1 M phosphate buffer (pH 7.5) containing 20% (w/v) glycerol and 1.4 mM mercaptoethanol, and sedimented again. The washed pellets, resuspended in I ml of O. I *M* phosphate buffer containing 30% (w/v) glycerol and 1.4 mM mercaptoethanol, were referred to as the microsomal fraction. They were stored for up to 1 week at - 80°C until used.

Enzymatic assays. Assay media for oxidative reactions contained 15% (w/v) glycerol and 0.7 mM mercaptoethanol, and microsomes (0.4-0.6 mg protein) in a final vol- ume of 200 µ.I. All enzymatic activities were carried out at 25°C as described in (6) with the following minor modifications.

The reaction mixture for ring-methyl hydroxylation and for N-monodemethylation of chlorotoluron routinely contained 10 kBq of [ca rbonyl-^{14}C]chlorotoluron (440 kBq/mmol) plus unlabeled herbicide to a fi- nal concentration of 500 µM. To evaluate the effects of the different pretreatments, enzymatic reactions were run for 25 min with 1.0 *mM* NADPH plus a NADPH regenerating system (6). The effects of agro-chemicals applied during incubations on activities of microsomal fractions from 2,4-D-pretreated cells were determined using 15-min runs in the presence of 0.5 mM NADPH. All assays were stopped by the

addition of 10 µl of 20% TCA. Unmetabolized chlorotoluron (30 to 40%) was partitioned into 0.5 ml n-hexane. The remaining radioactivity comprising herbicide and two metabolites was extracted with 90 µl cold acetonitrile.

Lauric acid in-chain hydroxylase activity was measured in a reaction mixture that contained *5* kBq of [1-^{14}C]lauric acid (2035 kBq/mmol) complemented with unlabeled lauric acid to a final concentration of 125 µM. NADPH concentration was maintained at 1.0 mM with the NADPH regenerating system. After incubation for 15 min, the assays were stopped with 10 µl of 20% TCA and 190 µl cold acetonitrile.

Cinnamic acid 4-hydroxylase activity was assayed using *5* kBq [3-^{14}C]trans-cinnamic acid (2035 kBq/mmol) and unlabeled cinnamic acid to a final concentration of 200 µM. Cofactor conditions were the same as for the LAH assays. Assays were stopped after 6 min with 10 µl of 20% TCA and 90 µl cold acetonitrile.

After sonication and centrifugation of the incubation media, substrates and metabolites of each enzymatic reaction were analyzed from two 100-µl samples by reversed- phase HPLC using a Nova-Pak C_{18} (Radial Pak) cartridge (8 mm x 10 cm) or a Zorbax-ODS column (4.6 mm x 25 cm). Each radioactive peak was separately collected and its level of radioactivity quantified by liquid scintillation (6).

Other methods. Quantitative determination of hemoproteins was carried out using the method of Omura and Sato (19). Microsomal proteins were estimated according to Bradford (20) with bovine serum albumin as a standard.

Effectors were added to the incubation media dissolved in the DMSO. Final sol- vent concentration was 1% (v/v) and did not affect enzymatic activities. Controls were supplemented with the same volume of solvent.

Results expressed in the figures are the means of independent experiments (n = 2 to 10) ± SD. Enzymatic activities and cytochrome P-450 levels are expressed in picomole/minute/milligram and picomole/milligram of microsomal proteins, respectively.

Results

Cytochrome P-450 contents and cytochrome P-450 activities in microsomes of wheat cells pretreated with various agro- chemicals. Cells were pretreated with 300 µM 2,4-D instead of 9 µMas usual, because this concentration stimulated *in vivo* chlorotoluron metabolism in wheat cells (5). Whatever its duration (24, 48, or 72 hr), this pretreatment resulted in an increase of the microsomal cytochrome P-450 content (Fig. 1). Whereas microsomes from control cells contained about 100 pmol cytochrome P-450/mg protein, 2.5-fold higher amounts were found after 48 hr of pretreatment. The pretreatment also affected the four cytochrome P-450-dependent activities examined. In control cells, activities of CPUH and CPUDM were almost constant throughout the experiment, LAH and CA4H activities decreasing slightly. Three of the enzymatic activities increased following 24-hr pretreatment, CA4H being an exception. As seen for cytochrome P-450 levels, stimulation reached a maximum after 48 hr. CPUH activity was less affected than CPUDM, with 2.9- and 4.7-fold stimulation, respectively. LAH and CA4H activities were increased 2.5- and 1.5-fold, respectively. Cytochrome *b5* contents and NADPH cytochrome *c* reductase activities were not affected by 2,4-D pretreatments (data not shown).

Effects of procloraz were estimated after 24-, 48-, and 72-hr pretreatments applied at 70 µM concentration, which provided the best

stimulation of degradation of chlorotoluron *in vivo* (21). Both 24- and 48-hr periods of procloraz pretreatment enhanced cytochrome P-450 contents (Fig. 2). However, the enzymatic activities were stimulated only by the 24-hr pretreatments. CPUH and CPUDM were stimulated more than LAH, which was enhanced 2-fold, and CA4H, which remained unchanged. After 48 hr, all activities showed rates comparable to those of control cells, or even reduced, as in the case of CA4H. After 72-hr pretreatments, the content of cytochrome P-450 was still enhanced, yet all enzymatic activities were lowered (not shown and Ref. (21)).

Effects of other agrochemicals added to the cell culture medium were evaluated after a 48-hr pretreatment (Table 1). A con- centration identical to that previously de- fined for 2,4-D (300 µM) was used for mecoprop. Because mecoprop belongs to the same herbicide family as 2,4-D, its effects were compared to those of the latter (Table I). Mecoprop and 2,4-D both enhanced cytochrome P-450 content to a similar degree. They stimulated CPUH and CPUDM activities, with a preferential effect on this latter. Moreover, they enhanced LAH and CA4H activities, mecoprop being especially active on the latter enzyme, which was increased 2.5-fold. Al- though 2,4-D and mecoprop stimulated the four oxygenation activities , 2,4-D had a slightly greater effect on the oxidation of chlorotoluron, whereas mecoprop stimulated more strongly LAH and CA4H.

The ability of chlorotoluron to induce its own metabolism was tested [a,] using 48-hr pretreatments at 200 µM concentration (Table 1). This value corresponded to a dose of herbicide which reduced the growth of cellular suspensions by 50% (21). In these conditions, cytochrome P-450 content was increased 2.9-fold. Chlorotoluron stimulated its own metabolism mainly through the CPUDM activity, but it only slightly

stimulated LAH and CA4H activities. No significantly greater effects were observed with 400 µM chlorotoluron pretreatments for 48 hr (not shown).

Three oxime ether safeners (cyometrinil, oxabetrinil, and CGA 133205) were tested for their ability to enhance the *in vitro* degradation of chlorotoluron at a concentration of 400 µM (Table 1). This was the concentration of cyometrinil that provided a maximal effect on wheat cells (5). After a 48-hr pretreatment, the total cytochrome P-450 content was 3-fold increased by cyometrinil, the other safeners being less active. The three safeners also stimulated the metabolism of chlorotoluron, again affecting preferentially CPUDM. LAH activities were comparable to those obtained with 2,4-D pretreatments. Only cyometrinil stimulated CA4H activity. Effects of cyometrinil could still be observed after 72-hr pretreatments, but they only concerned CPUH, CPUDM, and LAH activities (6).

Sensitivity of ring-methyl hydroxylation and N-demethylation of chlorotoluron to mfo effectors, herbicide safeners, and synergists. In this series of experiments, cells were pretreated with 300 µM 2,4-D for 48 hr. The activities of microsomes were assayed in the presence of 100 µM effectors. When added to microsomal preparations, the plant growth regulators tetcyclacis and paclobutrazol inhibited CPUH and CPUDM (Table 2). However, tetcyclacis preferentially inhibited hydroxylation by about 60%, with a significant effect at 10 µM, whereas paclobutrazol reduced both reactions to the same extent (45%). Procloraz was also an inhibitor of chlorotoluron metabolism. The morpholine fenpropimorph and the fungicide triazoles triadimefon and triadimenol were weak inhibitors of the hydroxylase, but were more active on the N-demethylation. PBO inhibited both activities by about 35%. Thus, these data indicate that the compounds differently affected the two

types of oxidation of chlorotoluron. Although procloraz and PBO did not discriminate between hydroxylation and N-demethylation, tetcyclacis and the triazoles affected preferentially the ring- methyl hydroxylation.

Herbicide safeners and synergists also inhibited cytochrome P-450-dependent activities (Table 2). At concentrations of 100 µM, inhibition amounted to about 20%, cyometrinil and tridiphane being the most active compounds.

Substrate specificities of chlorotoluron ring-methyl hydroxylase and N-demethylase. Substrate specificities of CPUH and CPUDM were tested using cells pretreated with 300 *µM* 2,4-D for 48 hr. Structural an-alogues of chlorotoluron appeared to have different effects on chlorotoluron metabolism when added *in vitro* at the same concentration as chlorotoluron (Table 2). Isoproturon, fenuron, metoxuron, and monuron were poor inhibitors. Other phenylureas, such as neburon and diuron elicited greater inhibitions. In alI cases, CPUH and CPUDM were affected almost equally. 2,4-D and chlorsulfuron applied at 250 µM had no significant effects on either of the activities (Table 2). The triazine herbicides were inhibitors of metabolism of chlorotoluron *in vitro,* the most active being terbutryne (Table 2). Conversely, diclofop specifically increased CPUDM 2-fold when added at 250 µM concentration (Fig. 3). An even greater effect of diclofop was obtained at 500-1000 µM, with a 3-fold stimulation of CPUDM activity. Even in these conditions, activities of CPUH remained unchanged.

Discussion

Levels of cytochrome P-450 and rates of chlorotoluron transformation *in vitro* were relatively low in microsomes prepared from control cells. This appears to reflect a general situation occurring in plants. The values presented here are comparable to, or higher than those generally reported for plant microsomal preparations. Our results equally show the necessity to use pretreatments in order to obtain reasonably active preparations from cultured cells. Similar conclusions were reached in recent *in vitro* studies on the involvement of cytochrome P-450 in detoxication of herbicides in plants (15, 17, 22). In wheat cells, pretreatments not only affected cytochrome P-450 levels and chlorotoluron metabolism, but also enhanced activities of LAH and CA4H. Nevertheless, rates of these latter two activities were lower in wheat than those obtained in other plant microsomes pretreated with the same inducers (23-25).

Generally, stimulatory effects of 2,4-D and cyometrinil on the metabolism of chlorotoluron in wheat cell cultures (5) were also found in this study using microsomal fractions from these cells. Ratios of activities of induced and control microsomes were in the same range of values as those reported for the *in vivo* activities of induced and control cells. This seems to indicate that induced activities detected *in vitro* corresponded to those reported *in vivo,* and are cytochrome P-450-dependent in the two cases.

Stimulations produced by 2,4-D administered as a pretreatment would not be due to an indirect toxic effect, because this chemical applied at 300 µM did not modify growth of cells (not shown). The time course

of stimulation showed a maximum at 48 hr. 2,4-D-enhanced cytochrome P-450 contents and unequally affected activities of various cytochrome P-450 enzymes, stimulating the oxidation of chlorotoluron to a greater extent than LAH and CA4H. It is likely that 2,4-D exerts an inductive effect in wheat cells. Stimulating effects of 2,4-D on the previous activities in other plant species have been already reported *in vivo* and *in vitro* (23-26). A similar interpretation could be applied to mecoprop.

Procloraz, applied as a 24-hr pretreatment, stimulated oxidation of chlorotoluron in wheat microsomes. This observation is in agreement with previous results showing that this fungicide stimulates chlorotoluron metabolism in wheat cells (21). It is possible that it acts in plants as in animals where it is a potent inducer of cytochrome P-450 monooxygenases (27, 28). It is worth noting that its stimulatory effects were transient, although the procloraz dose used was not toxic. This phenomenon is typical of biphasic agents such as nitrogenous or methylenedioxyphenyl compounds (29-32). *In vivo* administration of these compounds induces an initial phase of inhibition associated with the formation of a complex with cytochrome P-450s. A secondary phase involves *de novo* synthesis of cytochrome P- 450s and the generation of higher concentrations of the catalically inactive complex. Because the highest levels of cytochrome P-450 were detectable after 48 hr in wheat microsomes, procloraz might induce bio-synthesis of different isozymes exhibiting differential sensitivity toward the fungicide. It has previously been established that atrazine is able to stimulate its own degradation in corn (33). Our experiments show that chlorotoluron also stimulates its own metabolism in wheat, and that this increase of metabolism appears to be cytochrome P- 450-related. An induction of

cytochrome P- 450s and CA4H by monuron, a structural analogue of chlorotoluron, has been reported in Jerusalem artichoke tubers (23). Pretreatment of wheat cells with cyometrinil stimulated the metabolism of chlorotoluron *in vivo* (5) and *in vitro* (6). Thus, this safener appears to be effective in wheat because it also increased the metabolism of chlorsulfuron in this species (18). Because cyometrinil and its structural analogues did not exhibit any direct positive effects on oxidation of chlorotoluron when they were applied directly to microsomes, our results indicate that they may act as inducers of cytochrome P-450 monooxygenases.

In summary, cytochrome P-450- dependent activities exhibited different increases in response to the various pretreat- ments. In general, CPUDM activity was stimulated to the greatest degree, CPUH and LAH to a lesser extent, and CA4H was generally poorly affected.

Among the compounds tested for their capacity to interfere with the *in vitro* metabolism of chlorotoluron, special attention was paid to inhibitors of plant and mammal cytochrome P-450 monooxygenases (9-13, 34, 35). *In vitro* inhibitions of CPUH and CPUDM by plant growth regulators, sterol biosynthesis inhibitors, and PBO provide additional arguments indicating that both activities could be cytochrome P-450- dependent. In wheat cell microsomes, CPUH and CPUDM appeared to be only weakly sensitive to tetcyclacis, even at high concentration (100 µ.M). These results con- trast with the high sensitivity reported for maize cytochrome P-450 enzymes oxidizing bentazon, primisulfuron, and chorotoluron (22, 36, 37). As tetcyclacis is also a potent inhibitor of CPUH and **CPUDM** in *Veronica persica* microsomes (Polge *et al.*, in preparation), it is likely that the sensitivity of plant oxygenases to this fungicide differs according to the species.

The intermediate sensitivity to tetcyclacis of the diclofop aryl hydroxylase of wheat microsomes is in agreement with this hypothesis (15). PBO, another cytochrome P-450 inhibitor some- what inhibited the oxidation of chlorotoluron by wheat cell microsomes, while it showed a weak synergistic effect on plants (3). It is likely that this compound is weakly absorbed in wheat plants and/or that it undergoes a rapid inactivation.

Herbicide safeners had little effects on chlorotoluron metabolism in wheat, al- though it has been suggested that they could modulate cytochrome P-450 levels and their activities in corn by suicidal pro- cesses (38). Tridiphane, a synergist of the triazine herbicide atrazine in several plant species (39) and of the insecticide diazinon in the house fly (40), has recently been reported to synergize the activity of phenylurea herbicides (41). It has also been reported to inhibit cytochrome P-450 mono- oxygenases in mammals (42). That property might explain its synergistic activity with the phenylureas. Our report confirms the potential of tridiphane to inhibit cytochrome P-450 monooxygenases of wheat. A number of phenylurea analogues inter- acted with chlorotoluron metabolism when added to microsomes. Isoproturon exerted almost no effect, however, and since it is also metabolized in wheat by oxidative pathways, that suggests that its metabolism is not mediated by the same isozymes as those which metabolize chlorotoluron. Accordingly, it has been found that the tolerance of wheat to chlorotoluron and isoproturon is conferred by two different genes, which fits with the above hypothesis (Dr. J.W. Snape, personal communication). Herbicides degraded by cytochrome P-450 monooxygenases such as 2,4-D (14) and diclofop (15, 16), as well as herbicides metabolized *in viva* by wheat plants, e.g., chlorsulfuron (18), or by wheat cells, e.g., atrazine and terbutryne (17), were also used to study substrate specificities of the enzymes responsible

for the metabolism of chlorotoluron. Because 2,4-D and chlorsulfuron did not interact with chlorotoluron oxidation, their metabolism could be due to different isoforms of cytochrome P-450 or to another type of enzyme. By contrast, triazines strongly inhibited chlorotoluron metabolism, and it would be interesting to de- termine if they act as competitive inhibitors. The stimulating effect of diclofop is not yet understood. It may be comparable to the unexplained stimulatory effects produced by flavonoids (43) and polyamines (44, 45) on monooxygenase activities in liver microsomes. As diclofop caused *in vitro* a significant stimulation of CPUDM activity without a corresponding effect upon CPUH, this suggests also that different monooxygenases are responsible for the two activities.

These results, which report stimulatory and inhibitory effects of agrochemicals on chlorotoluron metabolism, are consistent with the hypothesis that at least two different cytochrome P-450 monooxygenases are involved in the metabolism of that herbicide in wheat cells. They also show that agrochemicals can affect cytochrome P-450 enzymes of wheat involved in physiological pathways or in the oxidative metabolism of herbicides.

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Table 1. Cytochrome P-450 Contents and mfo Activities of Microsomes Isolated from Wheat Cells after a 48-hr Pretreatment with Various Agrochemicals

Pretreatment conditions		Cyt P-450 (pmol/mg)	Specific activities (pmol/min/mg)			
			CPUH	CPUDM	LAH	CA4H
Control		100.1 ± 12.1	12.8 ± 0.5	9.5 ± 0.9	251.6 ± 13.2	2436.5 ± 60.1
2,4-D	(300 μ*M*)	246.2 ± 12.5	37.3 ± 2.1	44.7 ± 2.6	620.8 ± 20.0	3783.1 ± 264.0
Mecoprop	(300 μ*M*)	271.1 ± 21.5	28.3 ± 2.1	33.3 ± 3.6	655.2 ± 37.4	6151.5 ± 285.9
Procloraz	(70 μ*M*)	378.3 ± 45.0	12.1 ± 1.3	8.8 ± 0.5	322.8 ± 8.2	1510.6 ± 26.4
Chlorotoluron	(200 μ*M*)	285.5 ± 36.5	18.9 ± 5.0	32.4 ± 1.6	398.5 ± 56.1	2992.1 ± 26.7
Cyometrinil	(400 μ*M*)	315.9 ± 12.0	35.2 ± 0.1	48.3 ± 1.8	649.3 ± 5.8	4298.0 ± 205.2
Oxabetrinil	(400 μ*M*)	242.7 ± 22.8	33.0 ± 1.2	47.1 ± 1.5	598.9 ± 6.8	2529.0 ± 124.5
CGA 133205	(400 μ*M*)	218.9 ± 3.0	34.4 ± 1.3	47.3 ± 1.7	635.9 ± 26.5	2651.3 ± 234.0

Note. CPUH, chlorotoluron ring-methyl hydroxylase; CPUDM, chlorotoluron N-demethylase; LAH, lauric acid in-chain hydroxylase; CA4H, cinnamic acid 4-hydroxylase.

Table 2. Activities of Chlorotoluron Hydroxylase and N-Demethylase in the presence of Monooxygenase effectors in microsomes isolated from wheat cells that had been pretreated with 300 µM 2,4-D for 48 hr

Incubation conditions		Relative activities (% of control)	
		Hydroxylase	N-Demethylase
mfo effectors			
Control		100.0[a]	100.0[b]
Tetcyclacis	(10 µ*M*)	80.5 ± 3.1	89.1 ± 0.3
	(100 µ*M*)	38.1 ± 3.5	59.8 ± 3.2
Control		100.0[c]	100.0[d]
Paclobutrazol	(100 µ*M*)	57.0 ± 4.5	52.0 ± 2.3
Procloraz	(100 µ*M*)	54.2 ± 3.3	54.9 ± 3.0
Fenpropimorph	(100 µ*M*)	70.2 ± 1.6	66.6 ± 7.1
Triadimenol	(100 µ*M*)	76.7 ± 1.9	58.9 ± 2.5
Triadimefon	(100 µ*M*)	78.1 ± 2.4	47.5 ± 7.2
Piperonyl butoxide	(100 µ*M*)	63.0 ± 1.1	64.9 ± 5.2
Safeners and synergists			
Control		100.0[e]	100.0[f]
Naphthalic anhydride	(100 µ*M*)	93.1 ± 1.1	87.1 ± 4.6
Cyometrinil	(100 µ*M*)	78.7 ± 3.4	73.2 ± 3.9
Oxabetrinil	(100 µ*M*)	82.9 ± 7.6	79.7 ± 2.5
CGA 133205	(100 µ*M*)	82.0 ± 1.0	78.8 ± 1.6
Dichlormid	(100 µ*M*)	79.2 ± 3.6	87.7 ± 0.9
Tridiphane	(100 µ*M*)	77.5 ± 4.1	70.7 ± 6.6
Phenylurea analogues			
Control		100.0[g]	100.0[h]
Isoproturon	(500 µ*M*)	94.8 ± 4.6	99.1 ± 8.7
Fenuron	(500 µ*M*)	92.9 ± 5.6	85.4 ± 0.4
Metoxuron	(500 µ*M*)	86.0 ± 5.3	102.0 ± 4.6
Monuron	(500 µ*M*)	85.3 ± 4.8	84.4 ± 1.7
Monolinuron	(500 µ*M*)	71.9 ± 4.0	73.9 ± 1.6
Buturon	(500 µ*M*)	69.2 ± 4.2	68.0 ± 4.5
Monobromuron	(500 µ*M*)	65.8 ± 0.7	71.4 ± 0.8
Fluometuron	(500 µ*M*)	58.2 ± 3.4	61.6 ± 9.7
Chlorbromuron	(500 µ*M*)	47.9 ± 4.8	60.8 ± 2.6
Linuron	(500 µ*M*)	41.3 ± 2.5	41.3 ± 1.0
Neburon	(500 µ*M*)	39.7 ± 2.5	35.4 ± 2.9
Diuron	(500 µ*M*)	36.6 ± 0.5	26.7 ± 3.3
Other herbicides			
Control		100.0[g]	100.0[h]
2,4-D	(250 µ*M*)	92.8 ± 2.4	113.8 ± 4.7
Chlorsulfuron	(250 µ*M*)	86.0 ± 2.2	91.3 ± 4.9
Atrazine	(500 µ*M*)	67.8 ± 7.5	52.2 ± 3.4
Terbutryne	(500 µ*M*)	51.7 ± 0.5	41.8 ± 2.3

Note. Activities are expressed as % of control activities. The control values were: [a]44.4 ± 2.8, [b]55.3 ± 2.4, [c]44.9 ± 2.0, [d]55.6 ± 1.7, [e]45.8 ± 2.6, [f]56.2 ± 1.8, [g]41.1 ± 0.5, [h]50.7 ± 0.9 pmol/min/mg.

FIG. I. Cytochrome P-450 contents and mfo activities in microsomes isolated from wheat cells after 24-, 48-, and 72-hr periods of culture in presence of 9 µM (control, 0) or 300 µ.M 2,4-D **(e).** Abbreviations: CPUH, chlorotoluron ring-methyl hydroxylase; CPVDM, chlorotoluron N-demethylase; LAH, lauric acid in-chain hydroxylase; CA4H cinnamic acid 4- hydroxylase.

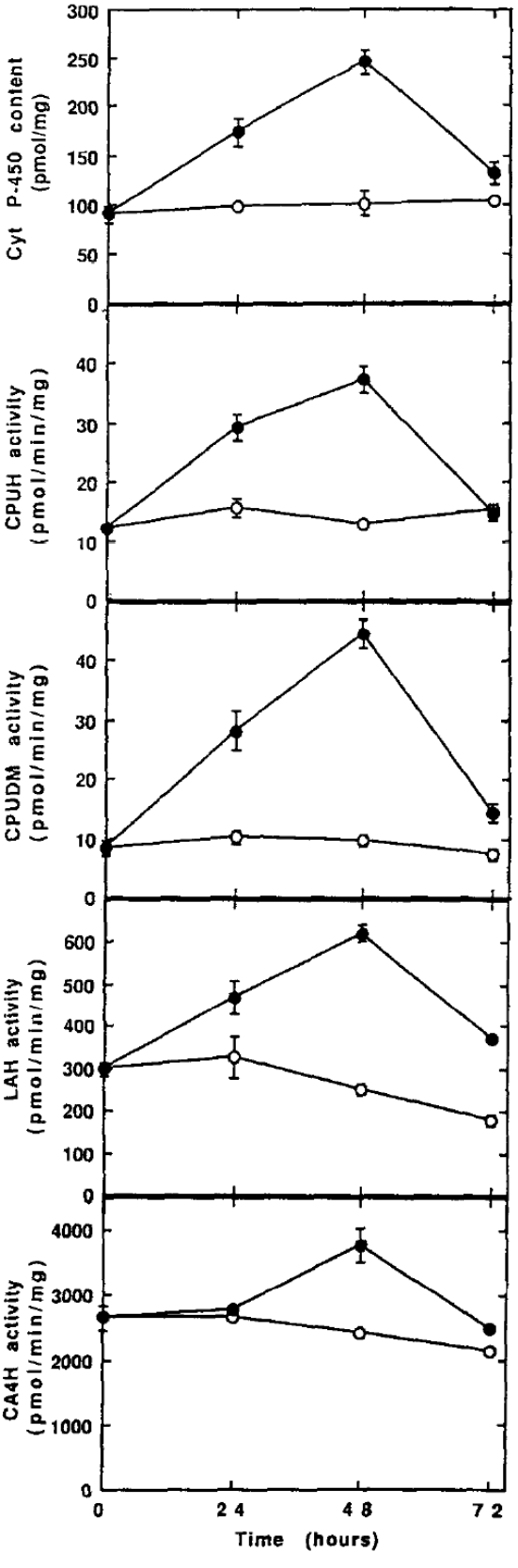
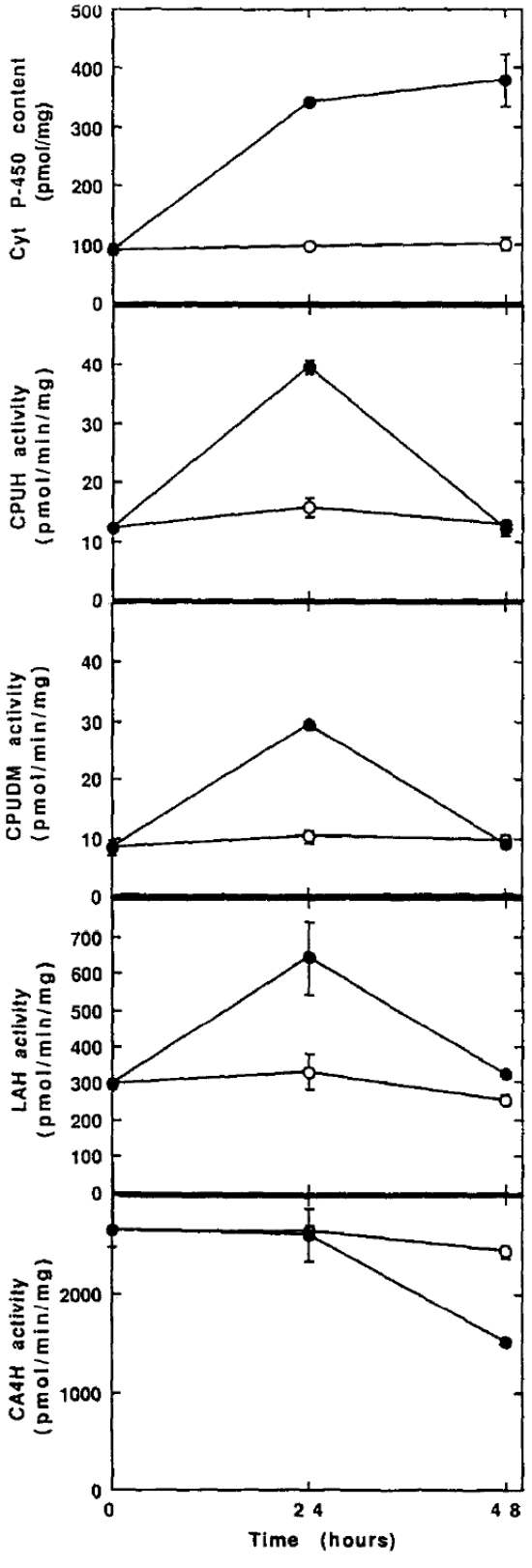

Cyt P-450 content (pmol/mg)
300
250
200
150
100
50
0
CPUH activity (pmol/min/mg)
40
30
20
10
0
CPUDM activity (pmol/min/mg)
40
30
20
10
0
LAH activity (pmol/min/mg)
600
500
400
300
200
100
0
CA4H activity (pmol/min/mg)
4000
3000
2000
1000
0
0
24
48
72
Time (hours)

FIG. 2. Cytochrome P-450 contents and mfo activities in microsomes isolated from wheat cells after 24- and 48-hr periods of culture without (0) or in presence (e) of 70 µM procloraz. Abbreviations: CPUH, chlorotoluron ring-methyl hydroxylase; CPUDM, chlorotoluron N-demethylase; LAH, /auric acid in-chain hydroxylase; CA4H, cinnamic acid 4-hydroxylase.


Cyt P-450 content (pmol/mg)
500
400
300
200
100
0
CPUH activity (pmol/min/mg)
40
30
20
10
0
CPUDM activity (pmol/min/mg)
40
30
20
10
0
LAH activity (pmol/min/mg)
700
600
500
400
300
200
100
0
CA4H activity (pmol/min/mg)
2000
1000
0
0
2 4
4 8
Time (hours)

F1G. 3. Activities of chlorotoluron ring-methyl hydroxylase (0) and N-demethylase (e) in presence of diclofop in microsomes isolated from wheat cells that had been pretreated with 300 µ,M 2,4-D for48 hr. Control values ■) were obtained during incubations without microsomal proteins or without cofactor.