Plant and fungal cytochrome P450's: their role in pesticide transformation

Christian P. Mougin

INRA, Unité de Phytopharmacie et Médiateurs Chimiques
Route de Saint-Cyr, F-78026 Versailles Cedex
email: mougin@versailles.inra.fr

Pesticides and Environment

Fate of xenobiotics in the soil

- Transfer of xenobiotics: Dr. V. Chaplain

- Biotransformation of xenobiotics: Dr. C. Mougin

keywords: bioavailability, biotransformation, bioremediation
filamentous fungi, enzymes, polymers, soil, xenobiotics

- Properties and functions of plant and fungal P450s
- Role of plant P450s in herbicide metabolism and selectivity
- Fungal P450s as tools for bioremediation
- Concluding remarks

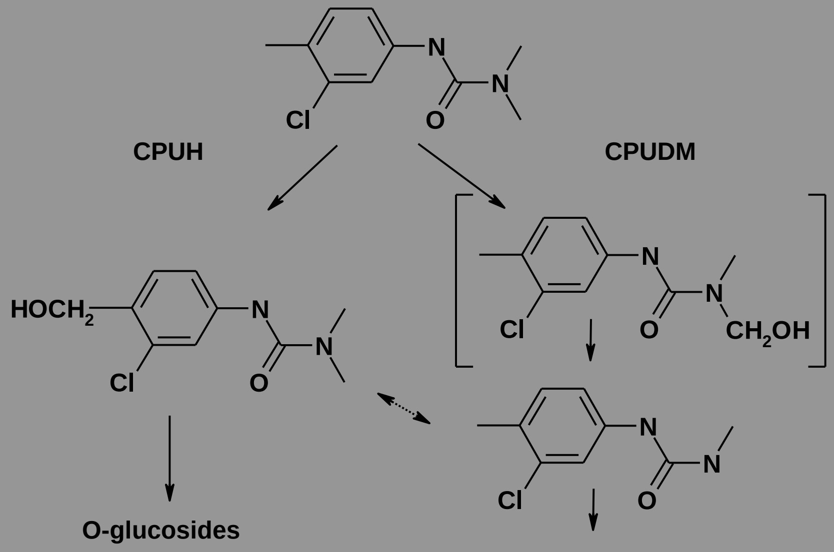
Plant and fungal P450s: properties

	Similarities		Divergences
Localization	Microsomes	F	soluble
Structure	P450 + CPR	F	P450-CPR
Cofactors	NADPH + O_2	P	ROOH
Induction	Physicochemical Physiological Xenobiotics	F	?
Inhibition	Haem ligands Mechanism-based	F	?

Plant and fungal P450s: functions

	Similarities		Divergences
Reactions	Hydroxylations Dealkylations		
Physiological substrates	Steroids n-Alkanes Fatty acids		
Xenobiotics	PAHs Pesticides	F	?

Chlorotoluron metabolization pathway in plants



2nd CPUDM

Effect of metabolism on chlorotoluron selectivity

	Wheat	*Veronica*	*Alopecurus*
Herbicide half-life	25 hours	6 hours	> 25 hours
Main metabolite	Hydroxylated	di-*N*-demethyl.	mono-*N*-demethyl.
Phytotoxicity	no	no	high

Effect of P450 effectors on chlorotoluron metabolism in wheat

	Inducers-Safeners	Inhibitors-Synergists
Whole plant		ABT Piperonyl butoxide
Cell cultures	2,4-D Cyometrinil Procloraz	ABT Paclobutrazol
Microsomes	2,4-D Cyometrinil Procloraz Chlorotoluron	ABT Piperonyl butoxide Procloraz

Inhibition of chlorotoluron metabolism by P450 inhibitors

Inhibitors		% of inhibition			
		Wheat		*Veronica*	
		CPUH	CPUDM	CPUH	CPUDM
CO		50	35	75	98
Tetcyclacis	10 µM	20	10	74	45
	100 µM	60	40	99	80
ABT	10 µM	74	57	0	0
	100 µM	100	100	35	7

Conclusions

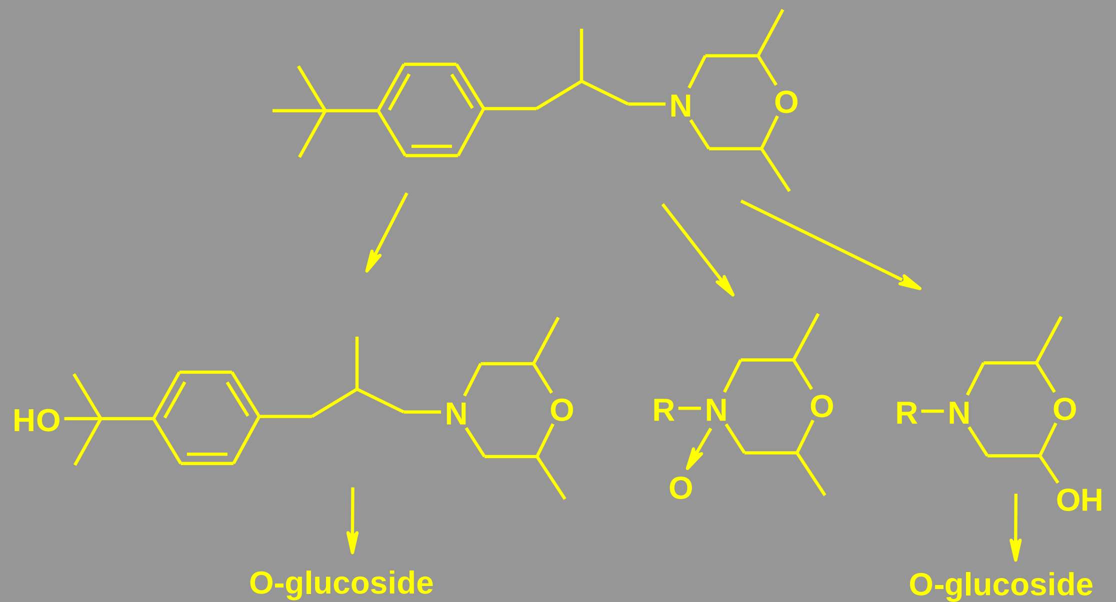
- Several P450s may be involved in the metabolism of a given pesticide within a single species

- Interspecific differences exist regarding P450 inhibition and induction in crops and weeds

- P450s play an important part in herbicide selectivity and resistance

- P450 could be targets to herbicide resistance engineering by chemical or genetic approaches

Fenpropimorph metabolization pathway in wheat



Effect of agrochemicals on wheat growth and sterol content

	Growth (%)	Sterols (%)		
		Normal	Abnormal	
Control	100.0	94.2	5.8	
Fenp.	65.2	9.2	90.8	
Fenp. + ABT	74.9	9.4	90.6	Accumulation in roots
Fenp. + Napht.	60.0	25.3	74.6	Depletion in leaves

Effect of agrochemicals on wheat P450 content

Agrochemicals	P450 (pmoles / mg)
Control (water)	113
Fenpropimorph	196
Fenp. + PBO	160
Fenp. + naphthalic anhydride	302
Fenp. + ABT	130
Fenp. + tetcyclacis	138
Fenp. + clofibrate	340

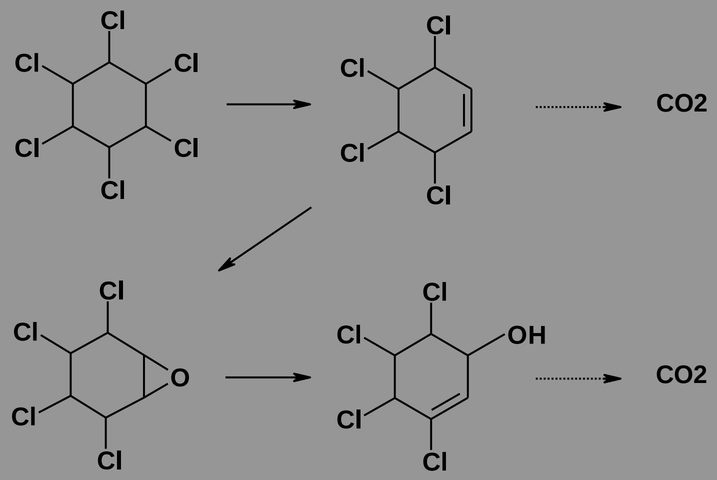
Involvement of P450s in fenpropimorph metabolism

- Fenpropimorph was converted to an oxygenated metabolite
- In microsomal fractions in the presence of NADPH
- Carbon monoxide bubbling inhibited the reaction
- Naphthalic anhydride and fenpropimorph itself stimulated the reaction

Conclusions

- The fungicide fenpropimorph can modify the growth and the sterol content of the host plant
- These adverse effects are modulated by agrochemicals known as P450 effectors
- The agrochemicals modify also the plant microsomal P450 content
- The fungicide is oxygenated by a P450 system

Lindane metabolization pathway in *P. chrysosporium*



Wheat bran pellets overgrown with *T. versicolor*

Effects of ABT and phenobarbital on metabolite and lindane contents in cultures of *P. chrysosporium* after 12-day incubations

	Relative abundance (%)				
			TCCOL		
	Unretained	Minor	TCCE	TCCH	Lindane
Controls	34.3	8.5	3.1	4.3	49.8
ABT (10^{-3} M)	11.6	0.0	0.0	0.0	88.4
PB (10^{-2} M)	37.9	1.1	5.2	0.0	55.8

Conclusions

- Metabolism of pesticides, such as lindane and atrazine, appears mediated by P450s in filamentous fungi cultured in liquid media

- Filamentous fungi can be easily inoculated into soils and well grow

- Filamentous fungi act as synergists of indigenous degraders

- Fungal efficiency remains limited by pesticide bioavailability

Concluding remarks

- P450s are widely involved in pesticide metabolism in plants and fungi
- An undestanding of the mechanisms regulating pesticide metabolism, with implications in herbicide selectivity, cross-resistance or bioremediation will require:

The isolation and functional characterization of the P450 encoding genes

and/or

The genetic engineering of already available genes

- Manipulation of P450 enzymes is a promising long-term challenge

Our partners

Plant P450s

R. Scalla, M.-F. Corio-Costet, F. Durst, D. Werck-Reichhart

Fungal P450s

M. Asther, P. Leroux