A SURVEY OF INRA STUDIES ON BIOBEDS

(INRA: *French National Institute of Agronomical Research*)

With the support of:

SEP · CONACYT MÉXICO

INRA
Institut National de la Recherche Agronomique

BAYER Bayer

THE INRA DEVICE

INRA DEVICE (2)

Tests of ecotoxicity

6 X substr. mixtures

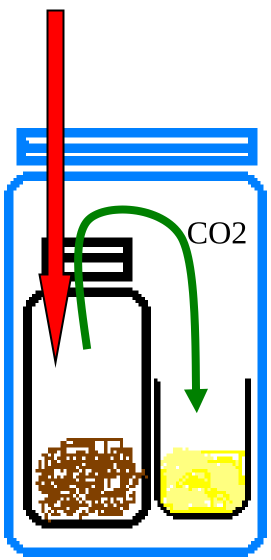
PPP wastes

Soil and residue analysis

treated and control vats (200 L)

(biobacs)

14C-PPP



Biomass size

Microbial degrading activities

BIOMIXES (v/v %)

VAT	SOIL	VERMICULITE	STRAW	COMPOST*	PEAT	PERLITE	TRAITEMENT
1	100%						Control
2	100%						Treated
3	50%	50%					Treated
4	50%	25%			25%		Treated
5	50%	25%		25%			Treated
6	50%	25%		25%			Control
7	50%	25%	25%				Treated
8	50%	25%	25%				Control
9	50%		50%**				Treated
10	50%			25%		25%	Treated

* a mixture of different peats +dolomie and sand

** around 45 g of straw per liter

Traitements appliqués sur les différents bacs entre octobre 2001 et octobre 2002

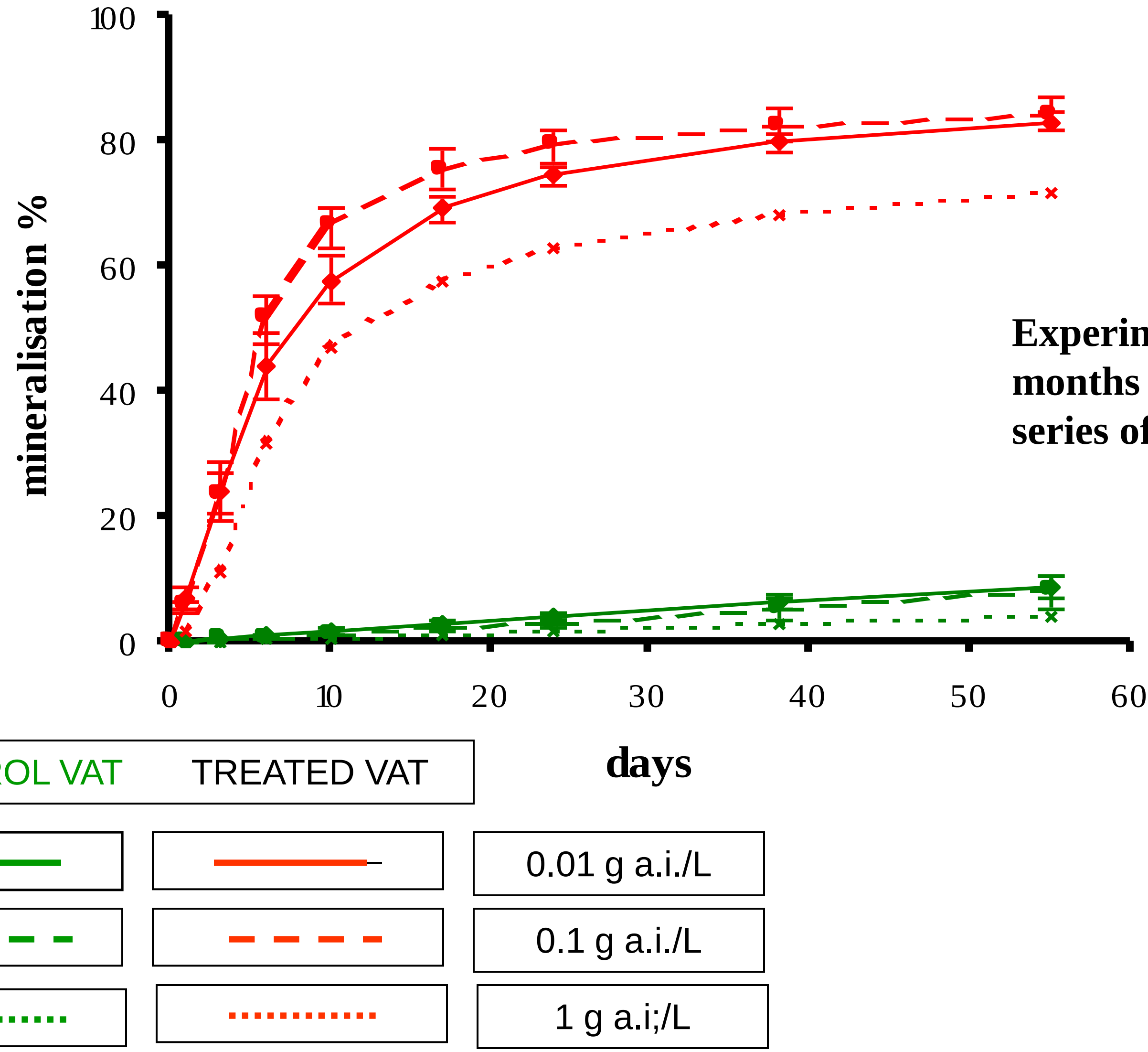
Active ingredient	October 2001: grams / vat	June 2002: grams / vat
Atrazine	2.0 g	-
Diuron	2.0 g	14.2 g
Isoproturon	2.0 g	8.0 g
Carbetamide	2.0 g	3.5 g
Chloridazon	2.0 g	19.5 g
Chlorpropham	2.0 g	0.1 g
Ethofumesate	2.0 g	5.0 g
Isoxaben	-	2.0 g
Phenmedipham	2.0 g	1.6 g
Metsulfuron methyl	-	0.2 g
Mesosulfuron	-	0.15 g
Glyphosate	2.0 g	14.4 g
Glufosinate	-	1.5 g
Terbuthylazine	2.0 g	14.2 g

SOME RESULTS…

1. **Effect of microbial adaptation**
2. **Effect of potential inhibitors**
3. **Effect of straw**
4. **Effect of Nitrogen**
5. **Effect of natural composts and manure**

Most of these studies were carried out using samples of treated or control vats. The samples were supplemented with different organic substrates and incubated for several months in laboratory conditions after or before treatments with 14Cpesticides.

Potential of diuron mineralisation strongly increased in pretreated vat *(similar results with atrazine and isoproturon but not glyphosate)*



Experiment carried out 7 months after the first series of waste addition

Biomixe :

Soil alone

About soil and biomixe adaptation:

Repeated treatments with certain pesticide increase the degradation potential of microflora.

It could be suggested to farmers to fill biobeds with adapted soil from their own fields

Many pesticides are now concerned by the phenomenon of microbial accelerated degradation but it could be interesting to focus on cometabolic examples of pesticide degradation.

Effect of potential inhibitors on the mineralisation of two herbicides

Xenobiotics (g/kg biomix)	Diuron (166 mg/kg) (minéralisation%)		Glyphosate (166 mg/kg) (% minéralisation)	
	10 days	55 days	10 days	55 days
Control	34	76	23	49
Cooper sulfate 0,16 g a.i./ kg	40	82	23	49
Cooper sulfate 1,6 g a.i./kg	30	78	20	44
Cuprosan 0,66+0,55+0,55ga.i./kg*	17	64	24	50
DNOC 1,66 g a.i./kg	7	34	23	50
Fongicide mix 0,57g a.i./kg**	32	72	24	47
Detergent All Clear 1,66ga.i./kg	22	72	21	47
Engine oil 16,6 mL / kg	26	73	23	51

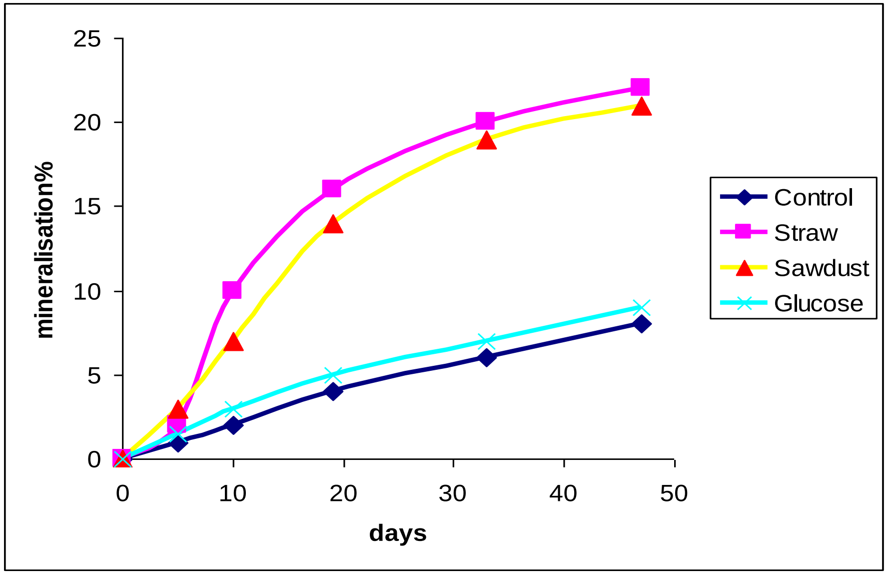
* cuivre+maneb+zineb

** iprodione+pyriméthanil+cyprodinil+ fludioxonyl+ fenhexamide

Biomixe : sol/verm./tourbe : 50/25/25

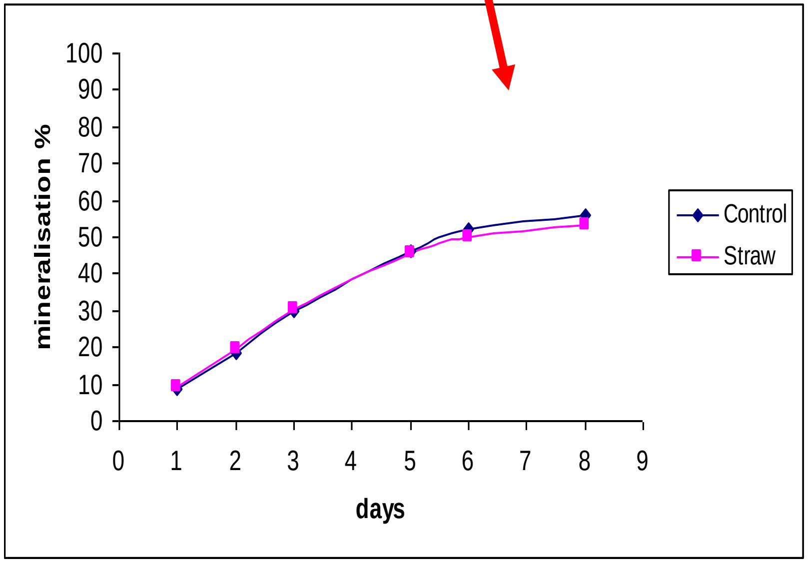
Effect of straw amendments on the mineralisation of 14Cethyl-atrazine:

Importance of soil adaptation and pesticide molecule



Cometabolic conditions: strong effect of straw added together with the pesticide

Adapted soil: no effect



Other pesticides (Initial rates of mineralisation immediatly or 7 months after straw addition)

No effect or very small increase in the mineralisation of glyphosate, bentazone or 14C ring isoproturon (adapted soil)

About straw…

In the lack of plants (herbicide waste problem), there is an evident need to supplement the biomixe with a source of carbon and energy especially to sustain cometabolic transformations.

But not actual proof until now!

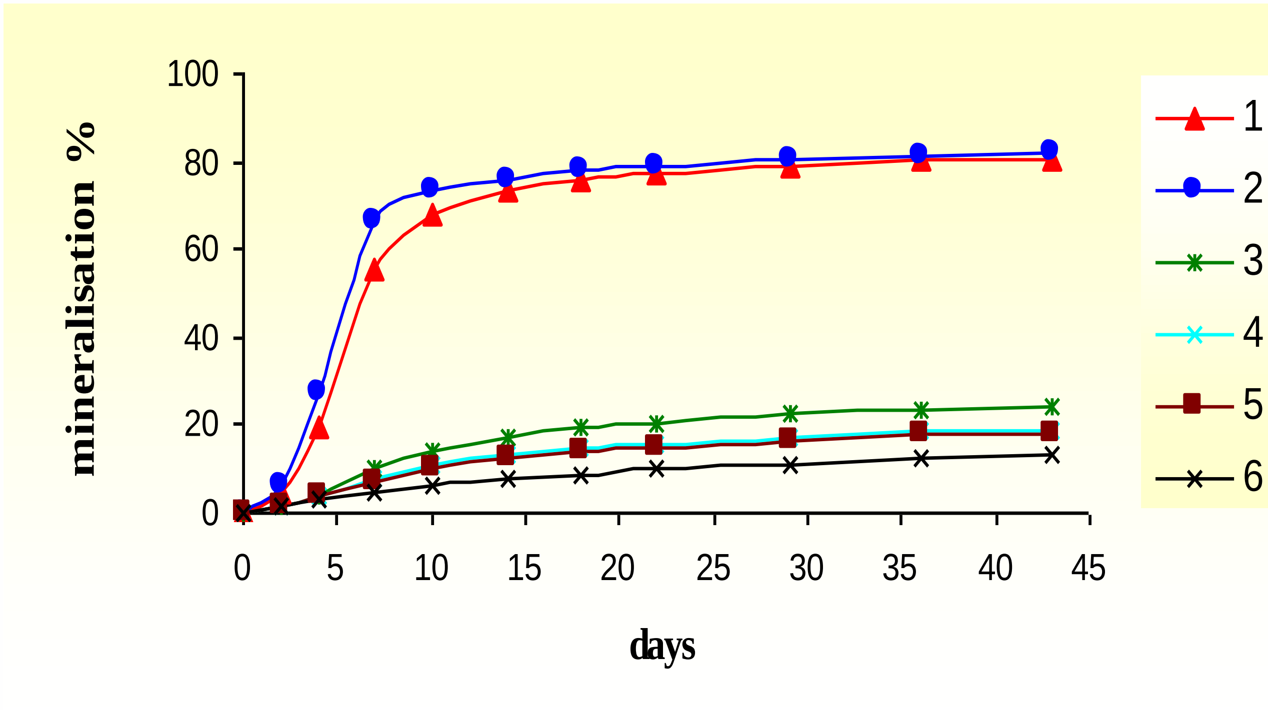
Why ?

Temporarly decrease in the biodisponibility of the pesticide trapped on straw fragments?

Choose other pesticide models (cometabolizing compounds)

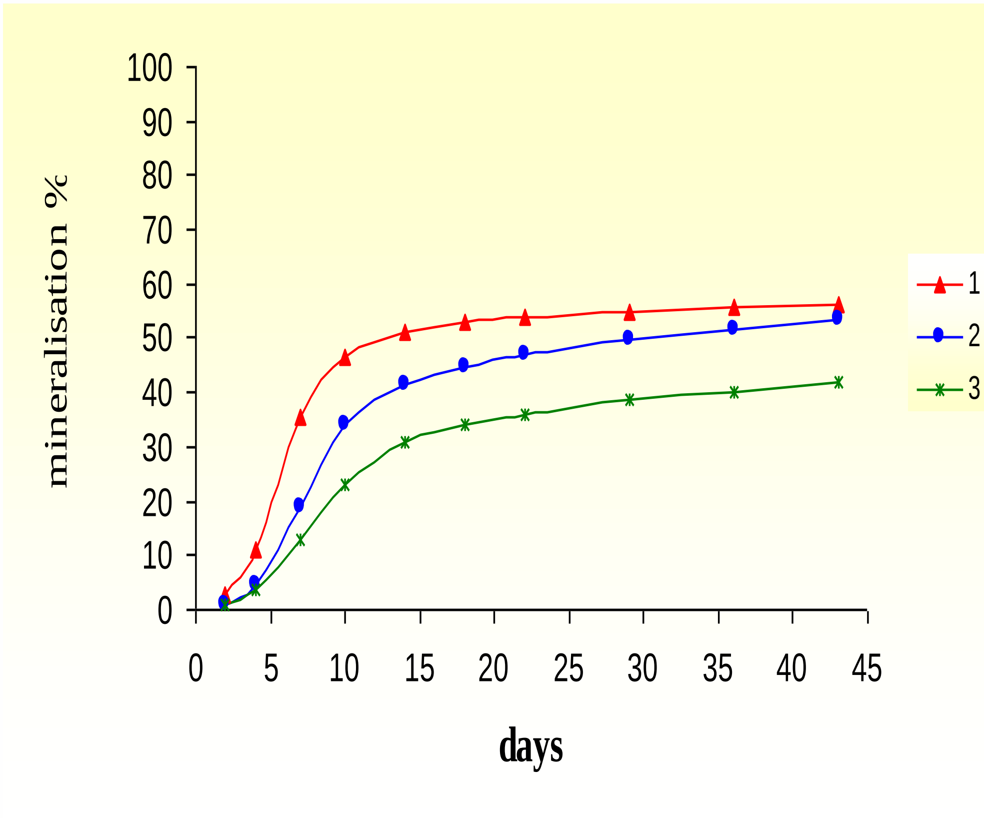
Need of long term experiments to induce a loss of biomass activity in the unamended soil?

Negative effect of peat on the mineralisation of 14C-atrazine …and other pesticides such as glyphosate



1. Soil 20 g ; water 5.6 ml
2. Soil 20 g ; water10.6 ml
3. Soil 20 g ; peat 2 g ; water 8.6 ml
4. Soil 20 g ; peat 2 g ; water13.6 ml
5. Soil 20 g ;peat 2 g ; water 18.6 ml
6. Soil 20 g ; peat 2 g ; water 28.6 ml

Nitrogen effect on 14C(ring)-atrazine mineralisation



1. Control without nitrate

2. ammonium nitrate (1.1 g "N"/kg sol).

3. ammonium nitrate (2.2 g "N"/kg sol).

Observations

1.For atrazine: a small increase in the initial rate of mineralisation was observed when the pesticide was added only 7 months after nitogen

2.An increase in glyphosate degradation was also observed in an experiment

Effect of natural composts and cow manure

Only few experiments were carried out…

Simultaneous biomix supplementation with manure often determine a decrease in the mineralisation rate (biodisponibility?)

But a significative increase in the initial rate of degradation of bentazone, atrazine and isoproturon but not glyphosate was observed 7 months after biomix supplementation with 5% cow manure (experiment in progress)

In the same conditions a natural compost (30%) stimulated the mineralisation of bentazone, atrazine and isoproturon but slightly inhibited glyphosate mineralisation.

ARE PREVIOUS RESULTS IN LABORATORY CONDITIONS CORROBORATED BY RESIDUE ANALYSIS IN THE DIFFERENT BIOBEDS?

Analysis were carried out 6, 14 (some compounds) and 18 months after the last treatment of vats with pesticide wastes.

« BIOBAC » Efficiency

Example of a 200 L vat filled with a mixture of 100 L soil and 100 L (4,5 kg) wheat straw

Activ. ingredient g/vat	Résidues after 2yrs	Activ. ingredient g/vat	Résidues after 2yrs
Atrazine 2.0	<LQ	Glyphosate 16.4	0.14%
Carbétamide 5.5	<LQ	Isoproturon 10.0	<LQ
Chloridazone 21.5	0.02%	Isoxaben 4.0	<LQ
Chlorprophame 2.1	<LQ	Metsulf.met. 0.2	<LQ
Diuron 16.2	0.01%	Mesosulf. 0.15	<LQ
Ethofumésate 5.0	0.52%	Phènemédip. 1.6	<LQ
Glufosinate 1.5	<LQ	Terbutylazine 16.2	2.3%

Total apports matières actives : 102.35g

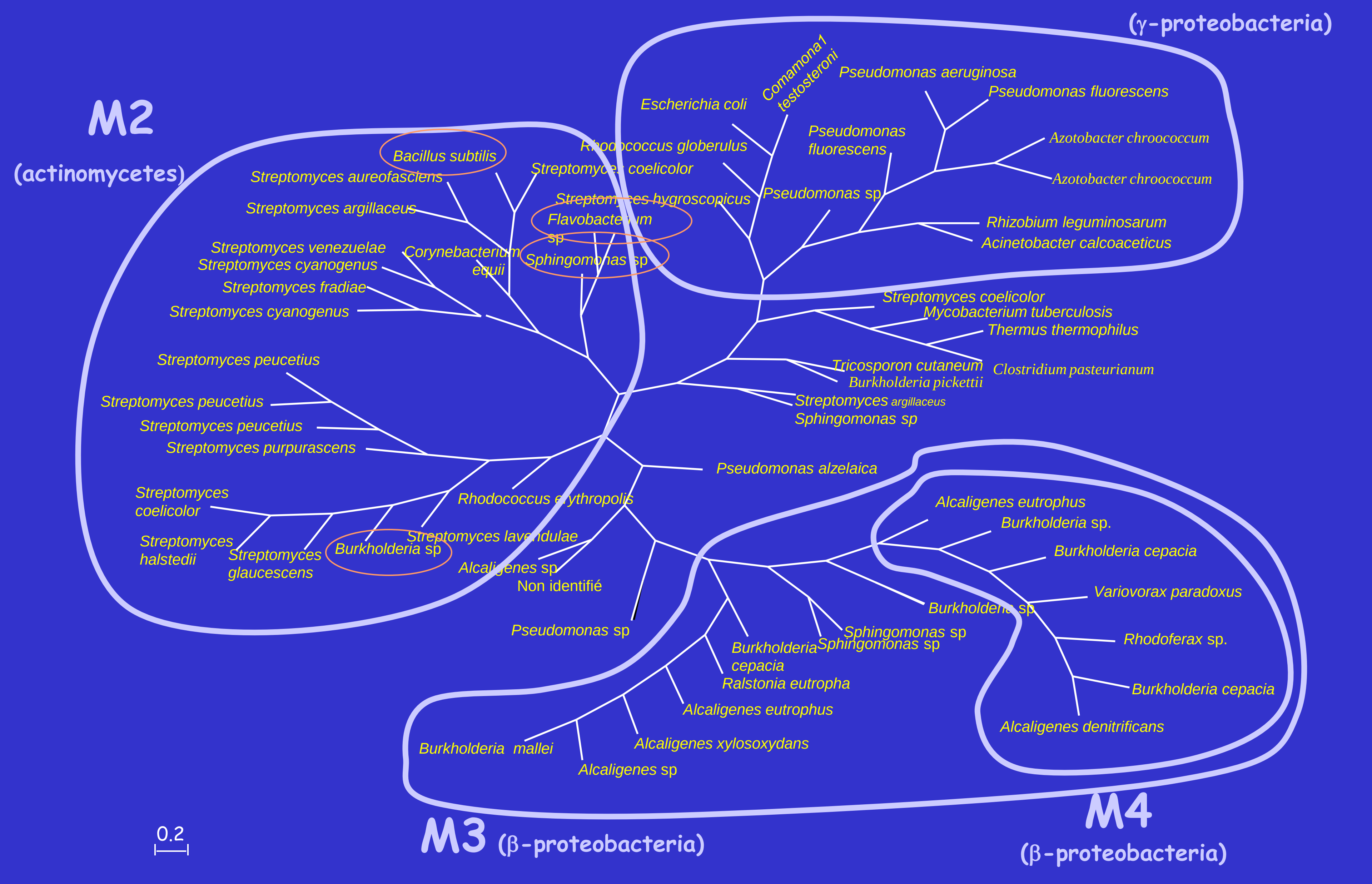
Total résidus après 18 months : 0.43g (hors métabolites) ; 0.52g (avec métabolites identifiés)

POSSIBLE EFFECT OF PESTICIDE RETENTION ON TOTAL RESIDUES OF TERBUTYLAZINE

(terbuthylazine residues: 5, 14and 18 months after last waste addition in biobed). ***(Total terbut. treatm.: 16.2g/vat)***

	mg/200 L	mg/200 L	mg/200 L
Biomix	**5 months**	**14 months**	**18 months**
Soil	2300	264	236
Soil/vermiculite	1980	87	173
Soil/verm/Peat	50	32	69
Soil/straw	4390	717	458

Phylogenic relationships of proteic sequences of bacterial monooxygenases

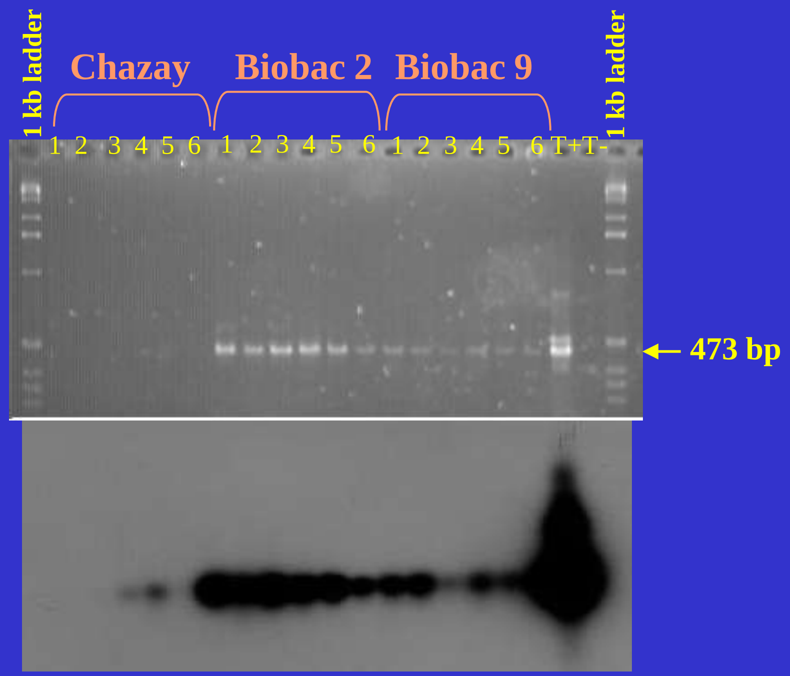


PCR amplification of monooxygenase genes directly from soil : analysis of the structure and the diversity of the hydroxylating bacterial community

-Design of degenerated primers pair from multiple alignment (M1, M2, M3 and M4)
-Amplification of these genes from DNA extracted directly from soil
-Hybridization to specific probes for confirmation

Amplification of M3 monooxyganses from soil DNA (Chazay, Biobac2 and Biobac9)

Hybridization of M3 PCR products to M3 probes



Evaluation of Biobac activity by quantitative PCR

On going PhD work : characterization of the structure and the diversity of bacterial monooxygenase communities by direct molecular approaches (PCR, cloning and sequencing)

OTHER STUDIES IN PROGRESS :

Two Investigations carried out in Burgondy on farmers practices of plant health products and « biobed feasibility »

Preliminary studies on residual toxicity of biomixes in case of spreading on field plots

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