Studies on the degradation of glyphosate by ligninolytic enzymes

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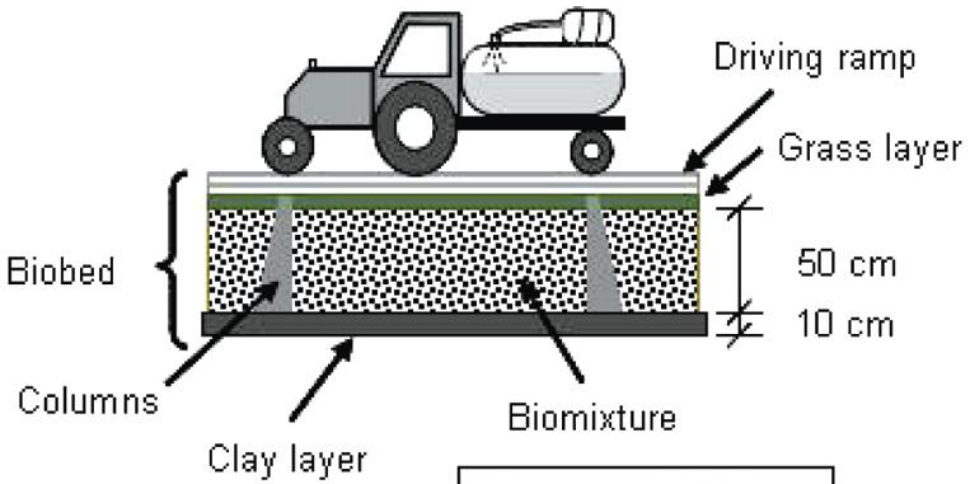
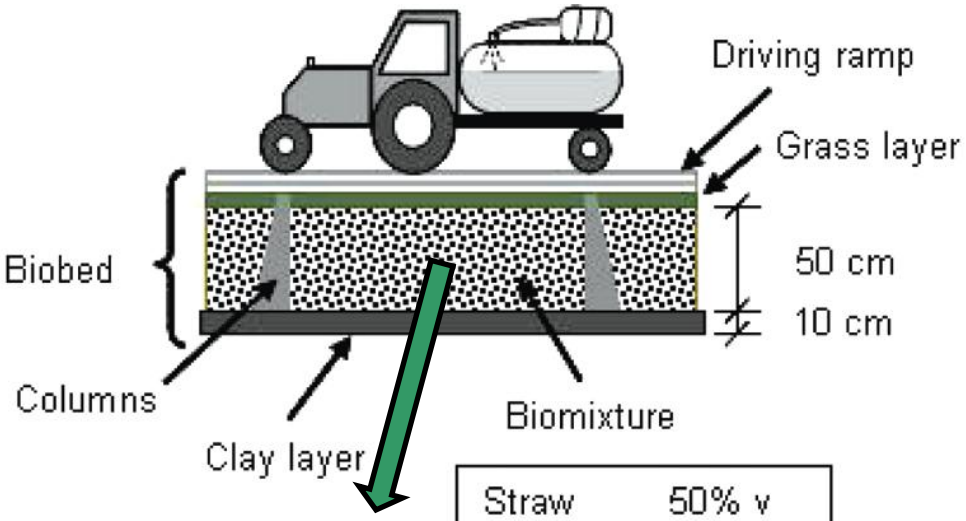
A Swedish Biobed



Straw	50% v
Peat	25% v
Soil	25% v

from Castillo *et al.,* 2008

A Swedish Biobed



Straw	50% v
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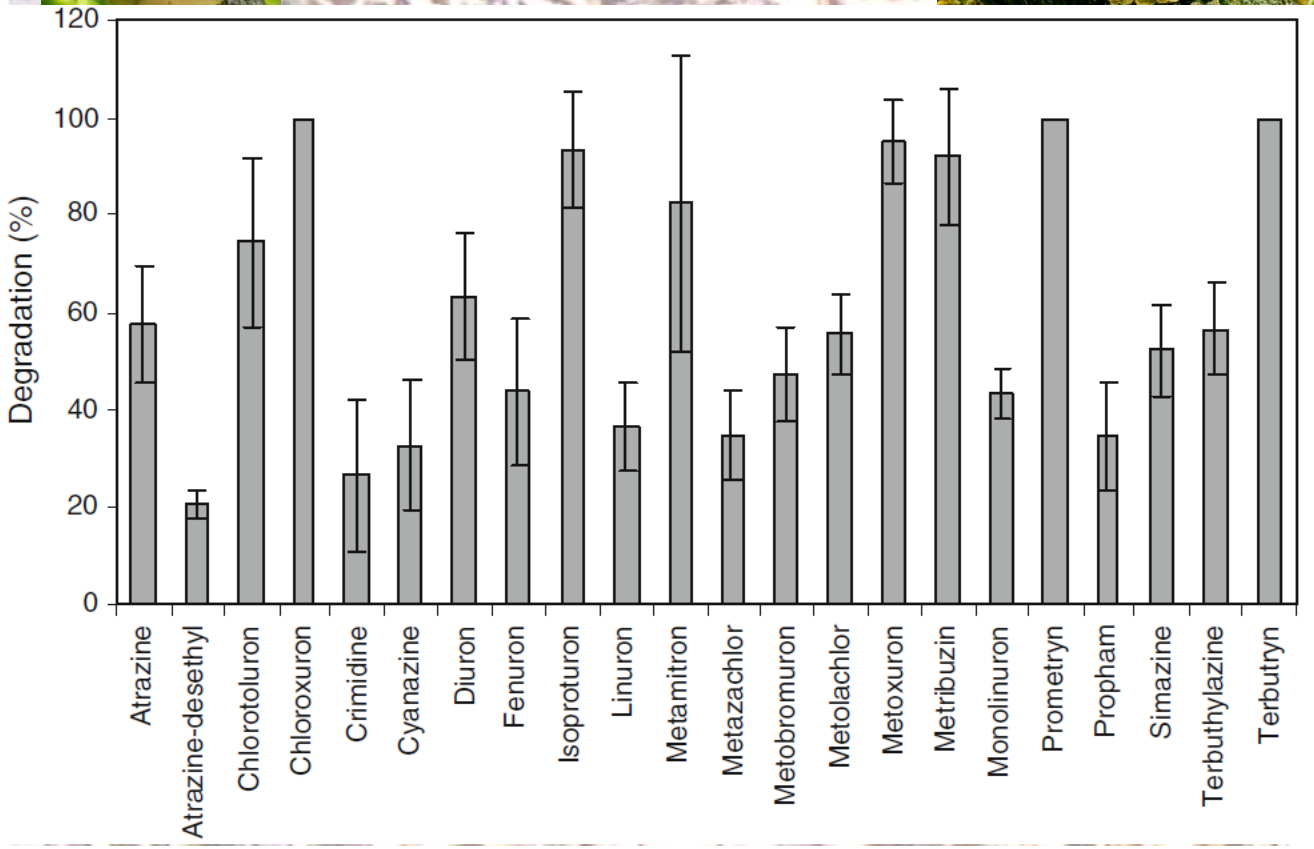
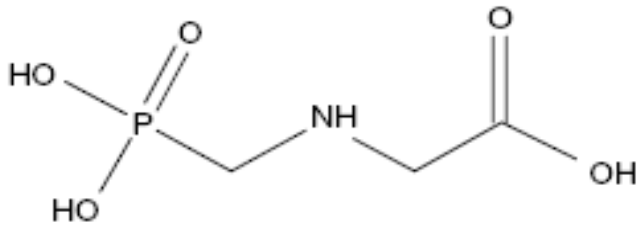
from Castillo *et al.*, 2008

white rot fungi

Lignin-degrading enzymes, lignin peroxidase, manganese peroxidase and laccase, produced by white rot fungi can degrade pesticides

Pesticide dissipation is correlated to phenoloxidases content.


Degradation (%)
120
100
80
60
40
20
0
Atrazine
Atrazine-desethyl
Chlorotoluron
Chloroxuron
Crimidine
Cyanazine
Diuron
Fenuron
Isoproturon
Linuron
Metamitron
Metazachlor
Metobromuron
Metolachlor
Metoxuron
Metribuzin
Monolinuron
Prometryn
Propham
Simazine
Terbuthylazine
Terbutryn



[*N* (phosphonomethyl) glycine]

Is glyphosate degraded by ligninolytic enzymes?

Microbial degradation of glyphosate

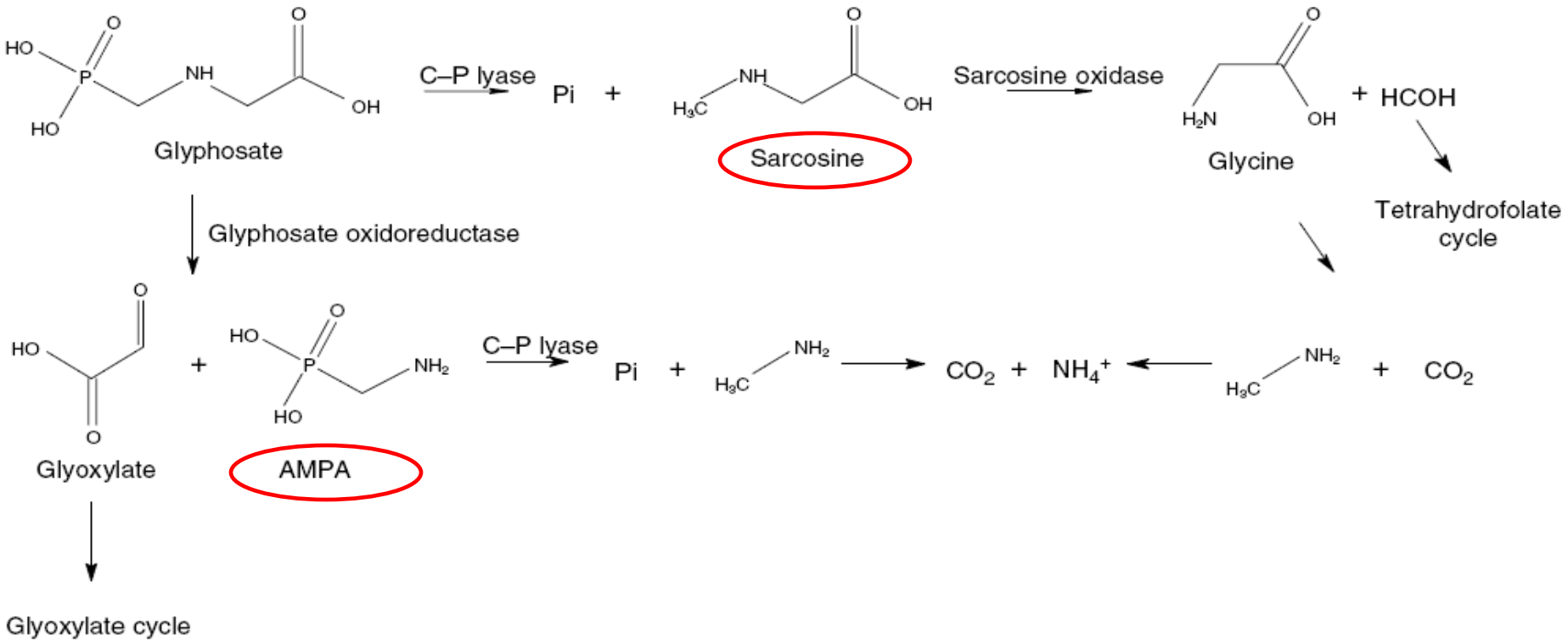


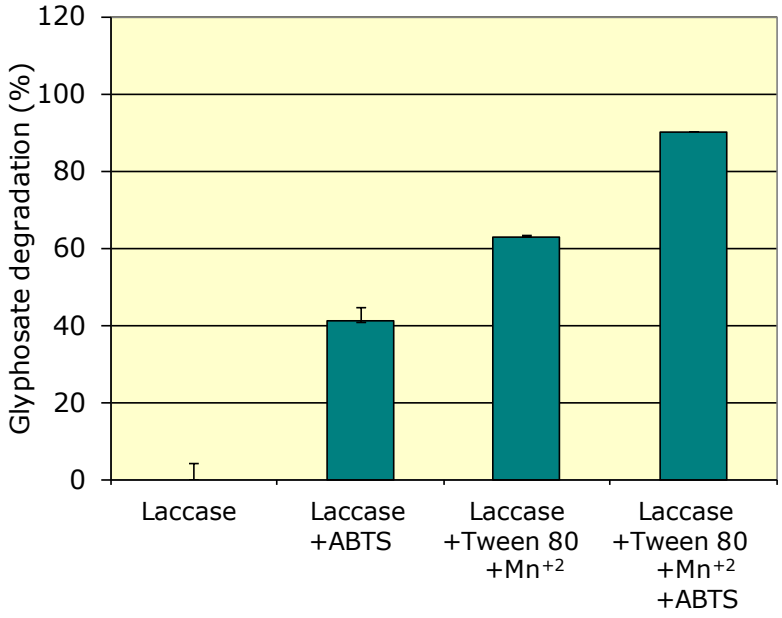
Figure 5. Microbial degradation of glyphosate through sarcosine or AMPA. Zwitterionic structure of carboxyl and amino groups omitted.

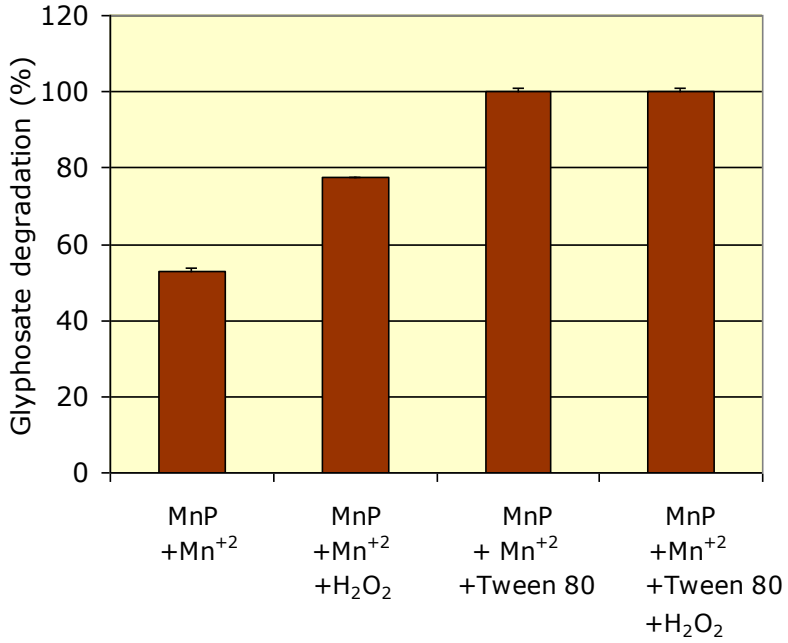
Borggaard & Gimsing 2008

In vitro experiments

Enzyme	Laccase	Manganese peroxidase (MnP)
Origin	*Trametes versicolor*	*Nematoloma frowardii*
Reaction mixture	Phosphate buffer Tween 80 $MnSO_4$ H_2O_2 ABTS laccase 0.15 U/ml glyphosate	Na acetate buffer Tween 80 $MnSO_4$ H_2O_2 MnP 1.5 U/ml glyphosate
24h incubation at 37ºC and 150 rpm. Glyphosate analysis by GC/MS		

Laccase and MnP degraded glyphosate within 24h





Pizzul *et al*., 2009

AMPA was detected in all cases where degradation of glyphosate occurred

Microbial degradation of glyphosate

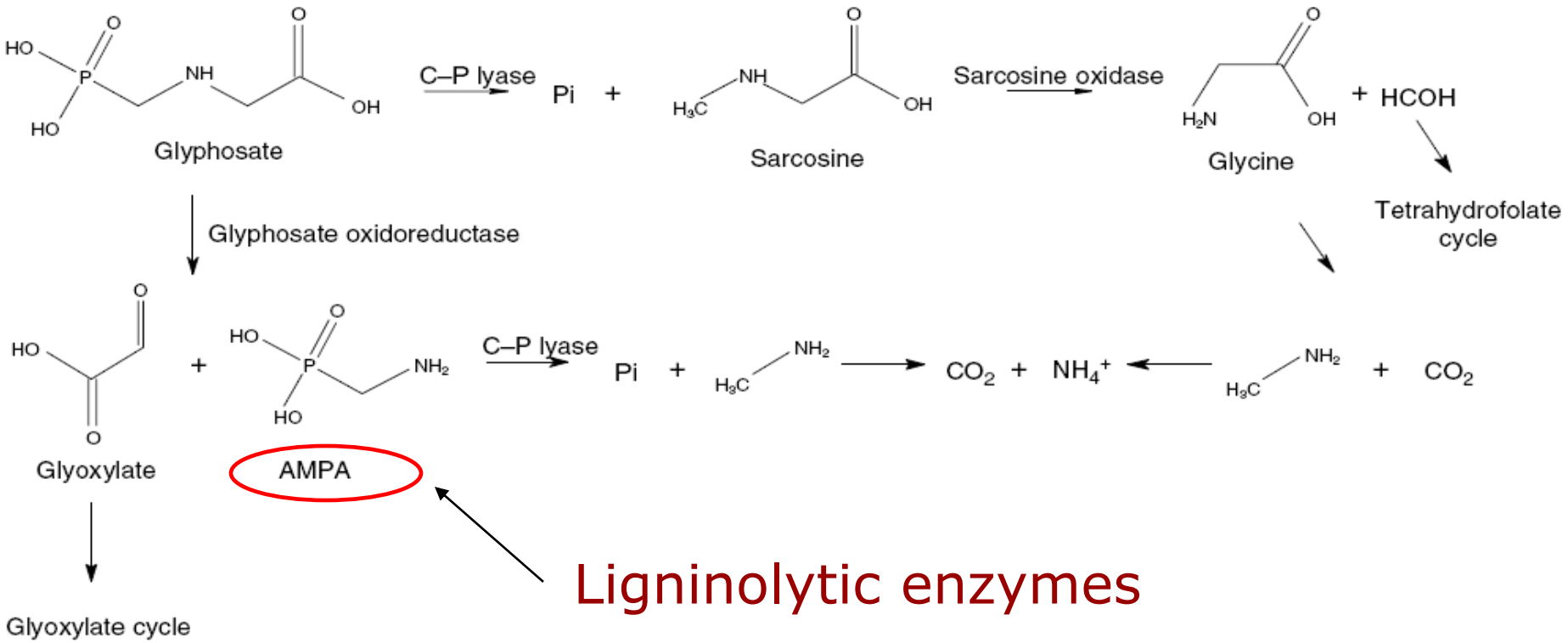


Figure 5. Microbial degradation of glyphosate through sarcosine or AMPA. Zwitterionic structure of carboxyl and amino groups omitted.

Borggaard & Gimsing 2008

But...

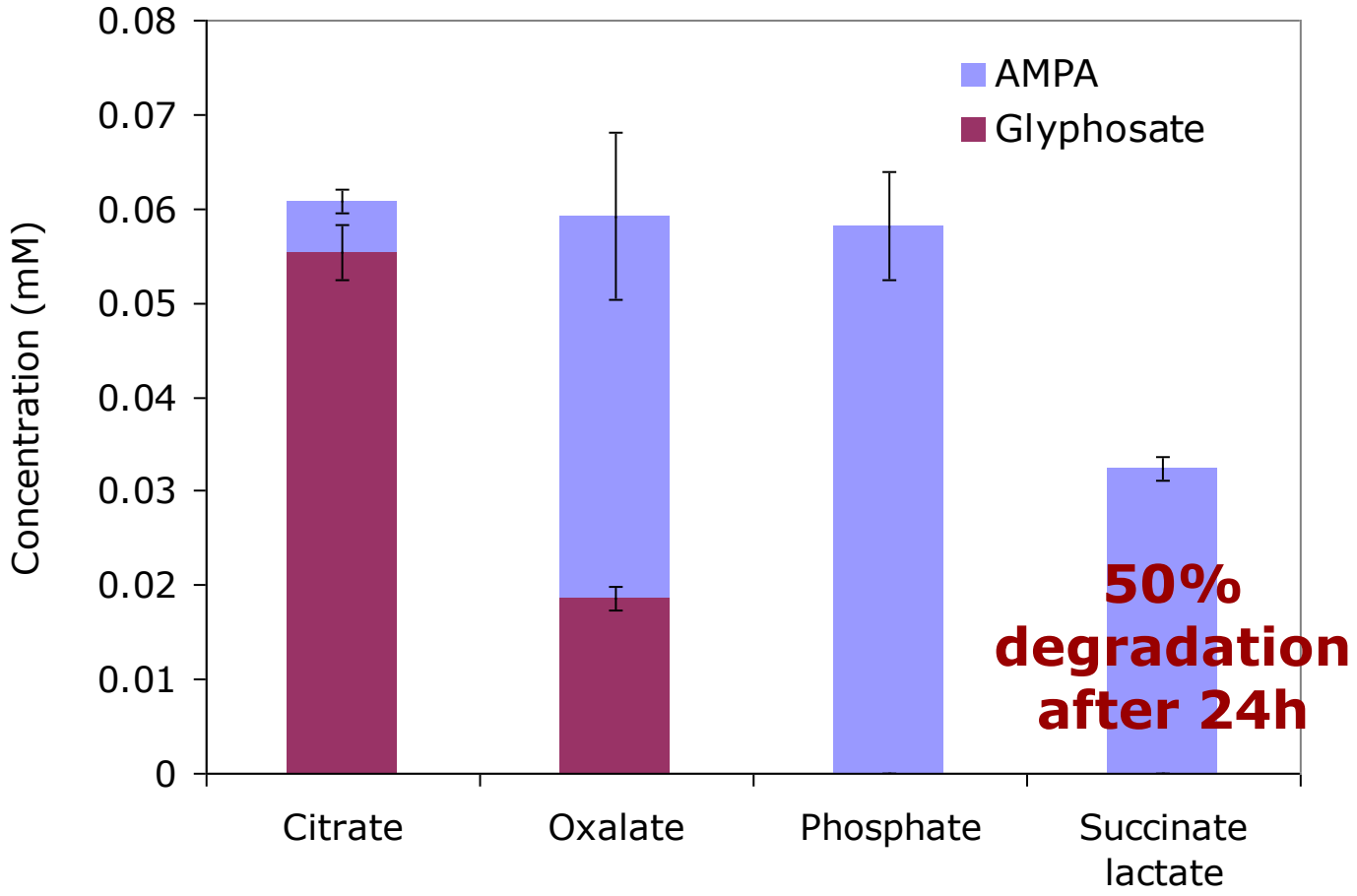
AMPA was not degraded and it accumulated

Is AMPA degraded by ligninolytic enzymes?

in vitro degradation of glyphosate by laccase

1. Buffer composition: citrate
   oxalate
   succinate lactate
2. pH: 4 to 7
3. Temperature: 10 to 50ºC
4. Presence of mediators

1. Buffer composition (pH 6)



2. pH (succinate lactate buffer)

Glyphosate was completely degraded in all the samples after 24h

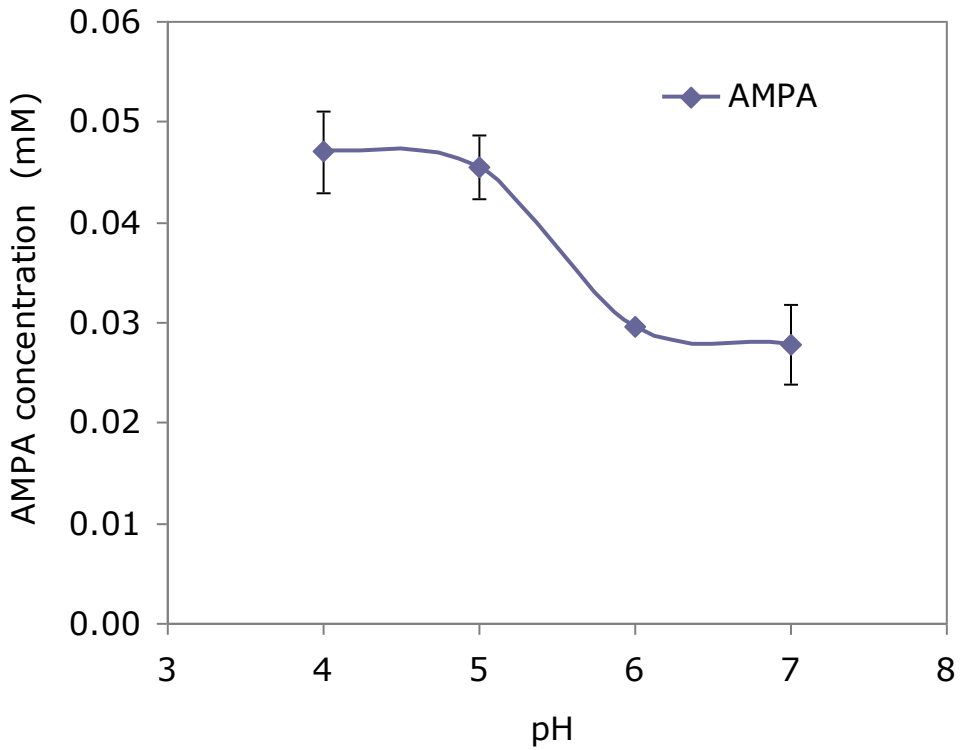
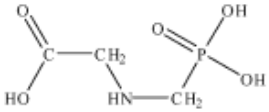
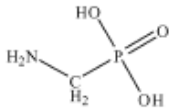
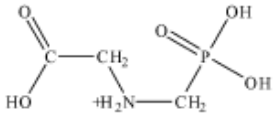
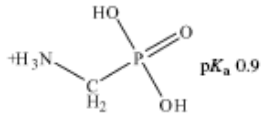
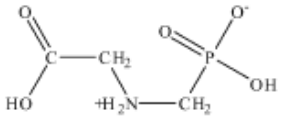
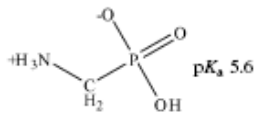
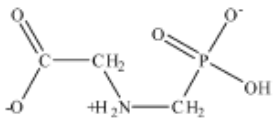
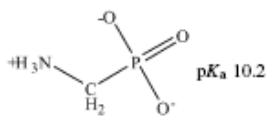
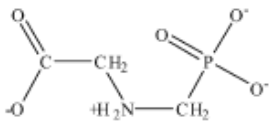
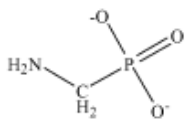
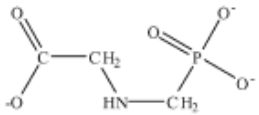


Table 1
Chemical structures, pK_a, and ionized species of glyphosate and aminomethylphosphonic acid (AMPA)

	Glyphosate		AMPA	
General structure				
Undissociated species	H_4A	 pK_a 0.8	H_3A	 pK_a 0.9
1st dissociated species	H_3A^-	 pK_a 2.3	H_2A^-	 pK_a 5.6
2nd dissociated species	H_2A^{2-}	 pK_a 6.0	HA^{2-}	 pK_a 10.2
3rd dissociated species	HA^{3-}	 pK_a 11.0	A^{3-}	
4th dissociated species	A^{4-}			

Piriyapittaya et al., 2008

Glyphosate and AMPA have four and three dissociation constants, respectively, becoming more negatively charged as pH increases.

Conclusions

Is glyphosate degraded by ligninolytic enzymes?

Yes, MnP and laccase, in the presence of mediators, can degrade glyphosate, with the formation of AMPA.

Is AMPA degraded by ligninolytic enzymes?

Yes, laccase can degrade AMPA, in the presence of organic acids and degradation is pH dependent.

In a biomixture:

- Ligninolytic enzymes degrade most pesticides studied
- Carboxylic acids and natural mediators (e.g. unsaturated fatty acids, syringaldehyde), which can promote pesticide degradation, are naturally present
- pH can vary among biomixtures. The addition of peat decreases the pH and therefore decreases "chemical availability" of glyphosate and AMPA.

The effect of pH on the growth of ligninolytic fungi has to be considered as well