Fungal degradation of the phenylurea herbicides isoproturon, diuron, linuron and chlorotoluron

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Main findings

• The soil fungus *Mortierella sp.* Gr4 transformed all studied phenylurea herbicides by dealkylation and hydroxylation reactions.

Background

The phenylurea herbicides isoproturon, diuron, linuron and chlorotoluron have been extensively used in agriculture. The herbicides are degraded by microorganisms in soil, but both parent compounds and metabolites have been observed in surface and groundwaters in concentrations exceeding the drinking water threshold limit of 0.1 µg l^{-1}.

The fungus *Mortierella sp.* Gr4 isolated from a Danish agricultural soil has previously been shown to demethylate and hydroxylate isoproturon.

Aims

• To investigate if the fungus *Mortierella sp.* Gr4 possesses a similar dealkylation and hydroxylation potential towards other phenylurea herbicides.

• To investigate the fungal transformation pathways and identify the metabolites produced.

Conclusions and perspectives

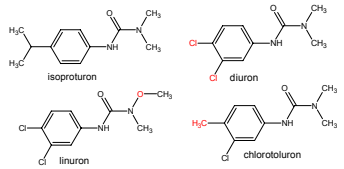
• *Mortierella sp.* Gr4 can perform several initial steps in the degradation of phenylurea herbicides. Both dealkylated and hydroxylated metabolites were observed.

• Linuron was the herbicide most rapidly transformed. The fungus performed both demethylation and demethoxylation in addition to hydrolysis of the carbonyl-bond yielding 3,4-dichloroaniline. Furthermore, a new linuron intermediate was observed.

• The methoxy-moiety of linuron might have a profound effect on the degradation when compared to the *N',N'*-dimethylated phenylurea herbicides.

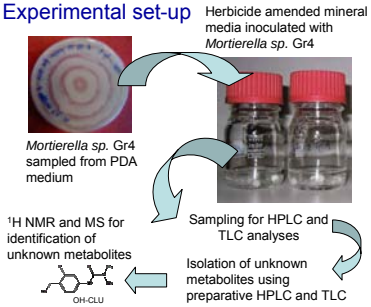
• Next step is to identify the linuron-intermediate by NMR and MS.

Phenylurea herbicides

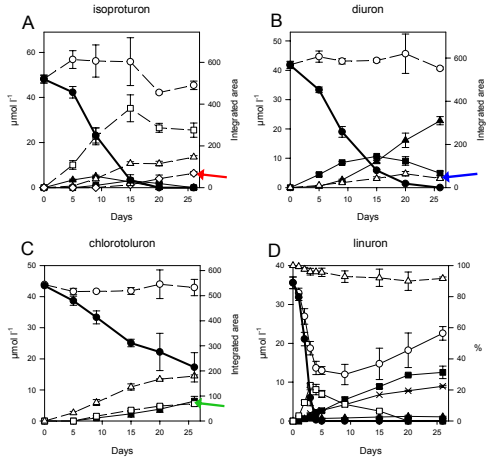


Chemical structures of isoproturon and the structurally related phenylurea herbicides diuron, linuron and chlorotoluron.

Experimental set-up



Degradation of isoproturon, diuron, chlorotoluron and linuron by *Mortierella sp.* Gr4 – HPLC data



Broken lines with open symbols can only be read on the right hand ordinate (integrated area) and represents hydroxylated and unknown metabolites.
-○- accumulated integrated area/concentration. n=3, mean + standard deviation.

Experiments were performed in liquid herbicide amended media inoculated with *Mortierella sp.* Gr4. Samples were analysed using HPLC/UV-VIS.

A)The fungus transformed isoproturon (-●-) under accumulation of several hydroxylated and demethylated metabolites and one new metabolite, presumably, 1-OH-DDIPU (red arrow).

B) Diuron (-●-) was transformed by demethylation to DCU. One unknown metabolite was observed but not identified (blue arrow).

C) *Mortierella sp.* Gr4 both demethylated chlorotoluron (-●-) to 3,4-CMU and a metabolite presumed to be a hydroxylated metabolite OH-CLU (green arrow).

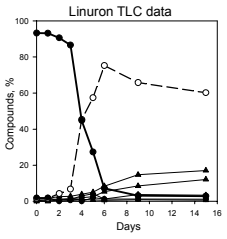
D) Linuron (-●-) was rapidly transformed. The concentration of the metabolites produced did not mirror the decreasing linuron concentration, which was reflected in the decrease of total accumulated concentration (-○-). Linuron was initially transformed to the aniline 3,4-DCA (-□-) and both the demethylated and demethoxylated metabolites DCMU and DCXU. The available linuron compounds in the medium is represented as % remaining ^{14}C-linuron (-Δ-).

Degradation of linuron by *Mortierella sp.* Gr4 – TLC data

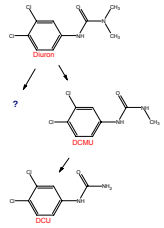
• The degradation of ^{14}C ring-labelled linuron by *Mortierella sp.* Gr4 was observed using TLC.

• Linuron (–●–) was completely transformed and the disappearance of compound was mirrored by accumulation of an yet unidentified metabolite (–○–).

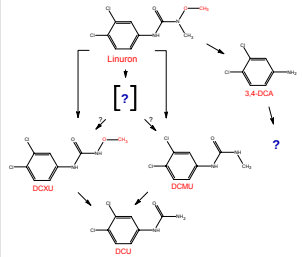
• The linuron metabolite has been isolated for further characterisation by ^{1}H-NMR and MS analysis.



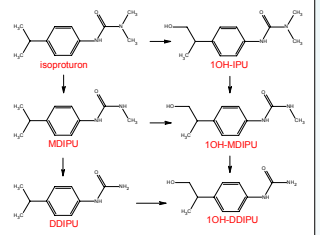
The proposed degradation pathway of diuron by *Mortierella sp.* Gr4



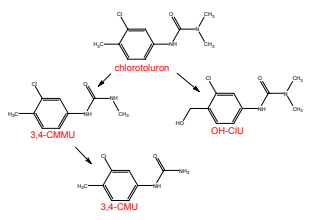
The proposed degradation pathway of linuron by *Mortierella sp.* Gr4



The proposed degradation pathway of isoproturon by *Mortierella sp.* Gr4



The proposed degradation pathway of chlorotoluron by *Mortierella sp.* Gr4



(Handouts)