Degradation of the pesticide isoproturon is associated with the proliferation of diverse *Sphingomonas* spp.

Shengjing Shi and Gary D. Bending
Warwick HRI, University of Warwick, Wellesbourne, Warwick, CV35 9EF UK

Introduction

- The phenyl-urea herbicide isoproturon (IPU) is one of the most widely used pesticides in Europe, and is a major contributor to surface- and ground- water contamination of agricultural catchments.
- Earlier work at Deep Slade field in Wellesbourne, Warwickshire, UK, indicated that within field spatial variation of IPU degradation rate resulted from interactions between IPU catabolising *Sphingomonas* spp. and soil pH.
- The aim of this work was to investigate the diversity of *Sphingomonas* communities contributing to spatial variability in IPU catabolism using *Sphingomonas* genus primers.

Materials & Methods

- Soil samples (labelled as B, C, D, E, F) were taken at 20m intervals along a high pH (7-7.5) area known to give rapid degradation of IPU (transect 1), and a low pH area (6-6.5) known to give slow degradation (transect 2), which were separated by 50m (Bending *et al*., 2003).
- An aqueous suspension of IPU was added to the soils to give a concentration of 15 mg kg^{-1} soil. Parallel control samples were set up using distilled H_2O in place of IPU. At intervals over 65 days, IPU residues were extracted from sub-samples of soil using acetonitrile, and concentrations were measured by HPLC.
- DNA was extracted from IPU treated and untreated soil samples at the point of 90% degradation (DT90).
- The *Sphingomonas* spp. community was amplified using the 16S rDNA primers Sphingo 108f/GC-40 and Sphingo 420r (Leys *et al*., 2004) and subsequently analysed by denaturing gradient gel electrophoresis (DGGE).

Results

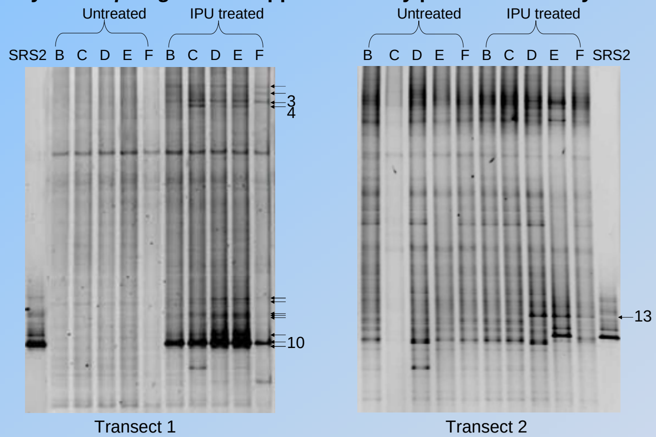
- Time to 50% degradation (DT50) ranged from 6.1 to 6.7 days in transect 1, and 8.4 to 25.7 days in transect 2 (Table 1).
- In samples from transect 1 and samples D, E and F from transect 2, degradation followed growth-linked kinetics with an exponential phase of degradation following a lag phase.
- In transect 1 and one sample from transect 2 in which degradation rate was rapid (E), IPU degradation was associated with appearance of up to 12 new bands, 7 of which matched those from isolate *Sphingomonas* sp. SRS2, which was isolated from Deep Slade field in an earlier study (Sørensen *et al*., 2001).
- In transect 2, growth-linked degradation was associated with the appearance of a band showing homology with *Sphingomonas mali,* which did not occur in samples from transect 1.
- There was no change to the DGGE profile in samples B and C from transect 2, which showed slow linear degradation kinetics indicative of cometabolism.

Table 1 IPU degradation data

	Transect 1					Transect 2				
Site	B	C	D	E	F	B	C	D	E	F
DT50 (days)	6.1	6.7	6.2	6.1	6.2	25.7	25.1	18.0	8.4	21.3
Degradation mode	GL	GL	GL	GL	GL	CM	CM	GL	GL	GL

GL: Growth-linked metabolism; CM: Cometabolism.

Fig. 1 Analysis of *Sphingomonas* spp. community profile at DT90 by DGGE



← indicates band appearing in samples treated with IPU.

Table 2 Sequence homology of bands cut from DGGE gels

Band No.	Closest match and Accession No.	Similarity (%)
3	*Sphingomonas* sp. CFDS-1 AY702969	99
4	*Sphingomonas* sp. D12 AB105809	96
10	*Sphingomonas* sp. SRS2 SSP251638	100
13	*Sphingomonas* mali SM16SR	98

Conclusions

- The specific strains of *Sphingomonas* spp. adapted to degrade IPU varied over a small area within a single field.
- In a high pH area, degradation was rapid, and was associated with proliferation of a variety of *Sphingomonas* spp. including a strain previously isolated from the field using enrichment techniques.
- In a low pH area, degradation rate was slow, and associated with proliferation of a lower number of *Sphingomonas* spp., including a strain which did not occur in transect 1.

References

Bending, G. D. *et al*. 2003. In-Field spatial variability in the degradation of the phenyl-urea herbicide isoproturon is the result of interactions between degradative *Sphingomonas* spp. and soil pH. Appl. Environ. Microbiol. 69:827-834.
Leys, N. M. E. J. *et al*. 2004. Occurrence and phylogenetic diversity of *Sphingomonas* strains in soils contaminated with polycyclic aromatic hydrocarbons. Appl. Environ. Microbiol. 70:1944-1955.
Sørensen, S. R. *et al*. 2001. Isolation from agricultural soil and characterization of a *Sphingomonas* sp. able to mineralize the phenylurea herbicide isoproturon. Appl. Environ. Microbiol. 67:5403-5409.

ACKNOWLEDGEMENT: We thank the UK Department for Environment, Food and Rural Affairs for funding.