Ecotoxicological testing of single species of soil organisms in the laboratory

Caul S[1], Griffiths BS[1], Thompson J[1], Birch ANE[1], Krogh PH[2].

[1]Scottish Crop Research Institute, Invergowrie, Dundee, DD2 5DA, UK

[2]Soil Fauna and Ecotoxicology Research Unit, National Environmental Research Institute, Vejlsoevej 25, P.O. Box 314, Silkeborg, DK-8600,Denmark

Project background and Introduction

The EU funded project Ecogen (www.ecogen.dk) was set up to evaluate the effect on non-target soil organisms of cropping systems including genetically modified (GM) crops and their associated agrochemicals. Maize expressing the *Bacillus thuringiensis* insecticidal protein (Bt) or herbicide tolerance (Ht) was grown in three Europen field locations, including Varois (France) and Foulum (Denmark).

We used single species and soil toxicological tests to assess the direct effects of Bt and Ht maize and relevant agrochemicals on non-target organisms, representative of the soil community.

Ciliate protozoa screened in microtitre plates

Tetrahymena pyriformis (pictured above) is a standard organism in ecotoxicology, but it is an aquatic protozoan, while *Colpoda steini* (pictured below) is a soil protozoan. Both were screened against agrochemicals used at the field sites.

Test compound	Protozoa: *Tetrahymena pyriformis*	Protozoa: *Colpoda steini*
$CuSO_4$ (Positive control)	Toxic	Toxic
Bt protein concentrate	No effect	No effect
Matrigon (herbicide)	No effect	No effect
Laddok TE (herbicide)	Toxic	Toxic
Lido 410 SC (herbicide)	Toxic	No effect
Skipper (molluscicide)	No effect	No effect
Carbofuran (insecticide)	To be done	To be done
Decis (insecticide)	To be done	To be done
Basta (herbicide)	Toxic	No effect

Table 1
Protozoan screening tests at concentrations expected in the field.

The protozoa were effected by the herbicides Lido, Laddok and Basta. The soil inhabiting protozoa *C. steini* was more resistant (only affected by Lido) than the freshwater *T. pyriformis*.

The nematode *Caenorhabditis elegans*

The reproduction of *C. elegans*, on agar plates with a bacterial food supply, in the presence of the test compounds at field concentration was evaluated after one week by comparison with control plates containing no test compound. Purified Bt protein was not tested in this system.

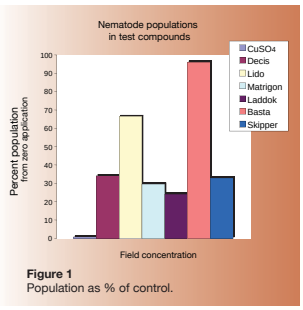


Figure 1
Population as % of control.

There was a definite reduction in the nematode population when in contact with the test agrochemicals. This was greater with the conventional herbicides (Lido, Matrigon, Laddok) than with Basta, glufosinate-ammonium, used on genetically engineered crops with glufosinate resistance.

It was difficult to replicate field application in this preliminary screening. Testing *C.elegans* against the compounds in a soil environment would be more representative.

Protozoan and Nematode population study in Varois and Foulum soils

To determine the effect of decomposing maize on the native populations of nematodes and protozoa, Bt maize (MEB307) and its near-isogenec control non-Bt maize (Monumental) were added to soils taken from two of the Ecogen sites. The soils were of different textures, with Foulum a loamy-sand and Varois a clay-silt soil.

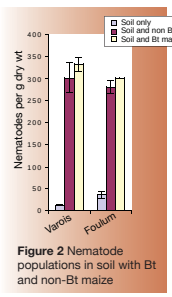


Figure 2 Nematode populations in soil with Bt and non-Bt maize

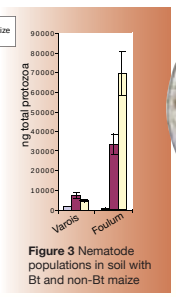
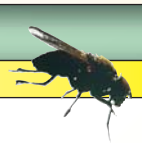


Figure 3 Nematode populations in soil with Bt and non-Bt maize

The populations of nematodes were not significantly different between the decomposing Bt maize and non Bt maize nor with different soil types.

There were more protozoa in Foulum than in Varois soil. The Foulum soil had significantly more protozoa in the Bt treatment than in the non Bt treatment.

Bt maize effects on a non-target soil insect



The Cabbage root fly (CRF) (*Delia radicum L.*) was used to determine any insecticidal effect on a non-target soil insect in a glasshouse pot experiment, using soils from two of the Ecogen field sites mixed with maize residues.

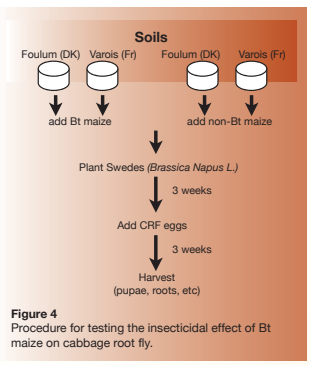


Figure 4
Procedure for testing the insecticidal effect of Bt maize on cabbage root fly.

There was no insecticidal effect on the non-target CRF, with equal growth of pupae in Bt and non-Bt ammended soil.

There was a soil difference with heavier pupae in the Foulum soil than in the Varois soil.

Conclusion and Future work

The agrochemicals tested had some deleterious effects on the single species tested, although these varied according to the organism under test. For example Matrigon had effects on nematodes but not protozoa at equivalent concentrations.

Further single species toxicology tests will be conducted on *C. steini* and *C. elegans* in de-faunated soil, using standard protocols to ensure comparability with fauna already tested (worms, enchytraeids, mites and Collembola) at NERI, Denmark.

The laboratory experiments showed no deleterious Bt-effect, either of the purified protein at concentrations far in excess of those measured or of added Bt-maize residues.

The single species tests, part of a tiered approach, will be compared to increasingly complex systems, mesocosm tests and field trials.

References

Griffiths BS et al, (2005) A comparison of soil microbial community structure, protozoa and nematodes in field plots of conventional and genetically modified maize expressing the Bacillus thuringiensis Cry1Ab toxin. Plant and Soil (2005) 273:135-146.

Griffiths BS et al, Soil microbial and faunal community responses to Bt maize and insecticide in two soils. Journal of Environmental Quality (2006) 35:734-741.

Acknowledgements

Ecogen is funded by contract QLRT-2001-01666 from the European commission.

SCRI receives grant-in-aid from Scottish Executive Environment and Rural Affairs Development (SEERAD)