

## Soil Nematode Field Study

### Advanced risk assessment on free-living soil nematode populations in natural environment



Nematodes from soil core sample  
(Source: ECOSSA GmbH, Starnberg)

Environmental risk assessments of plant protection products and in specific cases also of veterinary medicinal products take into account the effects of substances on soil organisms like e.g. free-living soil nematodes. If there are any concerns that the application of e.g. a nematicide could lead to unacceptable side effects on non-target nematodes it may be necessary to start with a higher tier nematode field study under realistic conditions as given by good agricultural practice to receive further data for the decision makers.

### Study Design

#### Test organisms

Nematodes, living in natural populations in the field.

#### Field Sites



The field study is normally carried out on an agricultural field, which should lie idle for several months. Each treatment group (e.g. control, 1 test item dose rate, reference item) includes 6 plots in a randomized block design. The whole field experiment and the individual plots are surrounded by buffer zones. Each plot has a size of 10 m to 10 m. The test item and the toxic reference item are applied with a movable plot sprayer or a movable granule applicator on bare soil according to good agricultural practice of the product. Afterwards the test item is incorporated into the soil. Grass, alfalfa or another crop is sown. The

study design can be easily adapted to the customer needs.

#### Nematode sampling and extraction

Soil nematodes are sampled with soil cores. 10 soil cores per plot are taken using a core sampler with approximately 5 cm diameter and 10 cm depth, each. After arrival at the laboratory the soil cores are subdivided into aliquots and filled into labelled containers. Each sub-aliquot is fixed by adding formalin containing rose Bengal to stain the soil nematodes for better recovery. Afterwards the Ludox centrifugation method is used to extract nematodes from soil samples.

One pre-sampling within 5 days before test item application and 4 post-application samplings after e.g. 1 week, 1, 4 to 6 and 12 months. Additional samplings can be carried out.

## Nematode counting and identification

The nematode suspension is obtained by centrifugation and is transferred into a nematode counting dish where the nematodes are counted using a dissecting microscope. 50 randomly selected nematodes from each soil sample are transferred in two subsequent mixtures of glycerol and ethanol (with increasing glycerol content) to slowly exchange body liquid of the nematodes with glycerol. After that, nematodes are mounted in suitably labelled permanent slides in glycerol. Nematodes are identified using a transmitted-light microscope (500 – 1000 x magnification). Nematodes are classified in different feeding types: Plant feeders, hyphal feeders, bacterial feeders, predators and omnivores. Nematode extraction and identification will be carried out by our partner ECOSSA (Starnberg, Germany).

## Endpoints

Total nematode density and feeding modes are determined at each sampling date for each treatment. The obtained data are analyzed using univariate (e.g. Williams test) and multivariate techniques (PRC). Additionally the Maturity index is calculated. This is a suitable tool for the data analysis. It is based on the classification of the roundworms to K- or r-strategists. MI allows drawing conclusions about the ecological status of a nematode community and may be considered as a measure of disturbance.

The nematode field study accepts initial test item effects and usually focuses on the timespan which is necessary to demonstrate recovery. Recovery is considered to have occurred when soil organism numbers are not significantly lower than the control on two consecutive sampling occasions.

## Guidelines and Literature

- Soil quality - Sampling of soil invertebrates - Part 4: Sampling, extraction and identification of soil-inhabiting nematodes (ISO 23611-4:2007); German version EN ISO 23611-4:2011

## About ibacon

Since 1994 the chemical and pharmaceutical industries trust in ibacon as a reliable partner for environmental risk assessment services and efficient performance of GLP-compliant studies as part of their regulatory projects.

## Contact



**Dr. Melanie Lichtenberger**

*Head of Testing Facility*

phone +49 6154 697-312

email [melanie.lichtenberger@ibacon.com](mailto:melanie.lichtenberger@ibacon.com)