Overview of the research focused on the application of entomopathogenic nematodes in Europe

Genetics for trait improvement in *Heterorhabditis bacteriophora*

- At the Volcani Centre (Israel) Differentially expressed genes (DEG) related to stress tolerance were detected in *H. bacteriophora* DXs guided by a previous *S. feltiae* transcriptome study conducted under stress conditions (Yaari et al. 2015)
- Conserved sequence regions were defined for each DEG by C. elegans sequence homology. Primers for qPCR were designed
- Gene expression was tested under two assays: i) evaporative desiccation (97% RH), and ii) hygroscopic desiccation (20% PEG) (Figure 1)

Results

- In *H. bacteriophora*, candidate genes were most highly expressed under evaporative desiccation after 16 hours, and under hygroscopic desiccation after 36 hours (Figure 2)
- A group of seven genes was selected as marker-set for further analyses assessing polymorphisms between stress-tolerant and -sensitive materials.

- At e-nema GmbH (Germany) *H. bacteriophora* accessions were characterized for their Di-longevity under oxidative stress
- A positive correlation has been found between Di-longevity and oxidative stress conditions (Yaari et al. 2015)
- In parallel, the individual *H. bacteriophora* accessions have been characterized for virulence. Interesting accessions with high longevity and virulence have been identified (data not shown)

Biocontrol of forest pests

- Pine weevil (*Hylobius abietis*) is a serious pest of reforestation throughout northern Europe. Weevils develop in tree-stumps and adults destroy young trees
- At the Forest Research Institute (Poland) EPN efficacy against the large pine weevil in clear-felled coniferous forests was assessed. (Figure 5)
- Parallel small- and large-scale field trials against the large pine weevil were conducted in Irish locations by the Maynooth University and Coillte in Ireland, and by the Forest Research Institute (Poland). Both EPN species resulted effective in field tests and showing considerable mortality of *H. abietis* larvae in treated stumps.
- In the work package Production technologies, the improvement of the downstream processing of *H. bacteriophora* is a main objective (e-nema, Germany). Two principles for separation of DJs from the culture broth were compared and a small-scale system was developed to monitor the shelf-life and virulence of stored EPNs after production.

The support by the EC to the BIOCOMES project will mark a milestone in the further establishment of EPNs as alternative control measure to chemical pesticides in Europe.

BIOCOMES: further development of entomopathogenic nematodes as biocontrol products

Within the EU-BIOCOMES project, the improved use of entomopathogenic nematodes (EPNs) in agriculture and forestry is a major interdisciplinary objective. The work package Genetics for trait improvement focuses on the understanding of the longevity and virulence traits in *Heterorhabditis bacteriophora*. Within this framework, predictive longevity bioassays were developed and natural isolates have been characterized and selected for longevity and virulence (E-nema, Germany). Additionally, desiccation-related gene markers were determined by RNA-seq and qRT-PCR validation (Volcani Centre, Israel). In parallel, differentially-expressed virulence genes were identified via RNA-seq (University of Azores, Portugal).

Within the work package Biocontrol of forest pests, the efficacy of EPNs against the large pine weevil (*Hylobius abietis*) in clear-felled coniferous forests has been assessed. Steinernema and Heterorhabditis EPNs were compared for their effect on pine weevils in roots and stumps. In addition, the effect of soil type and application method was analysed. Field trials were carried out by the Maynooth University and Coillte in Ireland, and by the Forest Research Institute (Poland). Both EPN species resulted effective in field tests and showing considerable mortality of *H. abietis* larvae in treated stumps.

In the work package Production technologies, the improvement of the downstream processing of *H. bacteriophora* is a main objective (E-nema, Germany). Two principles for separation of DJs from the culture broth were compared and a small-scale system was developed to monitor the shelf-life and virulence of stored EPNs after production.

The support by the EC to the BIOCOMES project will mark a milestone in the further establishment of EPNs as alternative control measure to chemical pesticides in Europe.

Conclusions

- Basic knowledge about the genetic basis of longevity and virulence in *Heterorhabditis bacteriophora* has been gathered
- A set of virulence- and stress-related candidate genes has been generated for *H. bacteriophora*
- Molecular markers associated to phenotypes of interest is a major priority task.
- EPNs applications show good efficiency against pine weevil in coniferous forests.
- New stress tolerant *H. bacteriophora* strains will be tested in field trials for crops protection aside clear-felled coniferous forests.

References:


WWW.BIOCOMES.EU