# First Annual Report (Abstract)

## 1. DESCRIPTION OF WORK

During the first year, the BIPESCO-team performed bioassays against scarab (*Melolontha melolontha*) and weevil (*Strophosoma* and *Otiorhynchus*) larvae to identify the most efficacious strains i.e.virulent (kill in as short as time as possible) and ecologically fit (persist well and retain virulence under field conditions). Protocols were developed for rearing of selected pest species to facilitate more extensive assays. Some bioassay procedures were standardised while others are being refined.

Physiological studies were conducted to learn more about attenuation of virulence. Inoculum (filamentous mycelium and conidia) from successive subcultures of different *Beauveria* and *Metarhizium* strains were assayed against insects to identify strains which lose virulence quickly. Enzyme profiles were prepared for some strains and successive subcultures partly for strain characterisation and partly to identify pathogenicity-related enzymes. In addition, secondary metabolites were analysed including putative pathogenicity determinants. Particular attention was focused on destruxins and oosporein.

Concomitantly, small-scale fermentation processes were run to optimise the production of fungal biological control agents (BCAs) in liquid- and solid state bioreactors. New formulation techniques to increase the shelf life of BCAs were tested and are still being developed. For example, compression of the formulation components (glidant, lubricant and anti-adhesive) and fungal inoculum directly after mixing. Progress was also made in the large scale production of *B. brongniartii* using a conventional diphasic fermentation step. More than 30 tonnes of fungal colonised barley kernels were produced and used in large scale field trials.

Protocols were developed for the molecular characterisation of fungal BCAs based on RFLPs of mtDNA, protease-gene differences and various amplified rDNA regions (18S, ITS-5.8S-ITS, 28S). These protocols are being refined to improve detection of *Beauveria* and *Metarhizium* at the species and strain level. Simple sequence repeat markers for B. brongniartii isolates were also developed for strain characterisation.

Knowledge of the spatial-temporal distribution of inoculum is essential for our understanding of how epizootics are initiated. For this reason, studies were conducted to determine the distribution and survival of *B. brongniartii* and *M. anisopliae* in the soil at sites where these pathogens had been used extensively for scarab and weevil control. Soil samples were taken, between April and September, from treated and untreated (control) plots to monitor the population dynamics of the pathogens and to provide information on the persistence of the pathogen by quantifying the degradation and recovery of the pathogen.

Dose-mortality studies were performed on benefical, non-target insects to learn more about the host range and potential impact of BCAs on these organisms

## 2. STATE OF PROGRESS

Subculturing did affect the phenotype and virulence of *Beauveria brongniartii* and *Metarhizium anisopliae*. Attenuation of virulence was manifest in two ways: (1) a gradual decline in virulence and (2) an initial decline and then a recovery. Most studies to date report a gradual or rapid decline in virulence with recovery only being achieved by passaging through an insect. Bioassays alone did not reveal the full variation in expression of attenuation but together with microscopy studies a considerable amount was learnt about the attributes of virulent isolates. For example, subtle differences were noted in the adhesion and the germination pattern of *M. anisopliae conidia* from different subcultures. This affected the overall mortality and LT50 value. This, together with changes in the Pr1 induction response in selective medium suggest that subculturing induces physiological changes in the fungus.

Development of appropriate formulations of conidia, mycelium and blastospores to guarantee viability and effectiveness of the BCA in the soil. Modifications were made to the coating equipment to ensure more uniform deposition of inoculum and formulants. An improvement was also made to the air supply system to reduce thermal stress of the final product. Oil-formulations were found to improve the efficacy of entomopathogenic fungi against insects. More work, however, needs to be done to elucidate how the oils improve the efficacy of the pathogen.

Recently developed primers can distinguish between species but specific primers will be designed to check for similarities/differences for all isolates in the BIPESCO collection. Studies are in progress examining the survival and distribution of *B. brongniartii* at old and new trial sites in Austria, Germany, Italy and Switzerland. Emphasis is being placed on the collection of soil samples from sites where inoculum had previously been applied to control *Melolontha*. Studies to date show that the pathogen can be isolated from areas where it has been applied or where *Melolontha melolontha* populations are dense (i.e. where there are natural epizootics). This relationship is being investigated in natural, undisturbed habitats. Soil samples were taken from untreated and applied sites (e.g. pasture, arable agricultural land, forestry, vineyards and orchards) to be able to study the edaphic effects (pH, soil texture, organic content, heavy metals, etc) on the persistence of the entomogenous fungi. In areas treated with *B. brongniartii* blastospores the densities and frequencies were below those treated with the commercial product. This is mainly due to soil applications of the fungus leading to a much higher fungal densities than treatment of swarming females, which subsequently inoculate the breeding areas.

# 3. ACHIEVEMENTS

The first milestone, establishing protocols to maintain and assay selected pest insects, has now been accomplished. The BIPESCO-team has defined appropriate methods for **(1)** conducting assays against target and non-target pests and **(2)** rearing of selected test insects. This has enabled the partners to evaluate, on the basis of the dose-mortality and time-dose response analyses, the virulence of their respective isolates. Furthermore, standardised bioassays were conducted in soil habitats to evaluate: **(1)** the ecological fitness of the fungal BCAs and **(2)** the effect of edaphic factors (soil type, moisture, temperature) on the efficacy of introduced pathogens. Eight highly virulent *Beauveria* and *Metahrizium* strains have been provided to each partner, to be able to compare specific isolates with the already characterised reference strains.

The entire rDNA gene-complex region (8.118 bp) from Metarhizium anisolpliae was sequenced in both directions and the sequences deposited in GENEBANK (AF 218207). The sequences are available to all the partners on request.