

BIPESCO SECOND ANNUAL REPORT

(INTERIM-REPORT 4)

1. OBJECTIVES

The objectives of the RTD project FAIR6-CT98-4105 (BIPESCO) according to the original version of the Technical Annex are unchanged. Regarding future actions the BIPESCO team will keep to the schedule and will achieve the goals outlined in the Technical Annex.

2. DESCRIPTION OF WORK

During the second year, the BIPESCO-team performed bioassays against scarab (*Melolontha melolontha*, *Phyllopertha horticola*) and weevil larvae (*Strophosoma* and *Otiorhynchus*) using *Beauveria brongniartii* and *Metarhizium anisopliae* isolates provided by the partners. Standard protocols were developed for rearing of selected pest species to facilitate more extensive assays. Bioassay procedures were standardised and statistical analysis of bioassay data refined.

Physiological studies were continued to provide more invaluable information on attenuation of virulence. Cultural conditions were shown to influence shelf life of inoculum and virulence. In addition, studies on fungal instability helped identify spontaneous mutants. A combination of biochemical and molecular studies showed that mutants of *Metarhizium anisopliae* BIPESCO 6 lacked the subtilisin genes Pr1A and Pr1B. These mutants had undergone considerable re-organisation of the genome. Bioassays showed that these mutants were less virulent than parent wild type strains thus confirming that Pr1 was an important virulence determinant. These mutants will undoubtedly act as excellent tools for the study of fungal virulence and help accelerate identification of other virulence genes. Studies are underway to determine the frequency of mutation as this could radically alter the composition and, subsequently, the quality of the inoculum.

Carbon utilisation patterns and enzyme profiles were prepared for selected strains and some subcultures partly for strain characterisation and partly to identify pathogenicity-related enzymes and metabolites. All eight BIPESCO-reference strains were morphologically characterised in submerged culture by describing all stages of growth (i.e. from germination of conidia to the stationary growth of hyphal bodies and filamentous mycelium).

Development of formulations that enhance virulence and increase the shelf life of the inoculum is an important component of this project. New formulation techniques to increase the shelf life of biological control agents (BCAs) were tested. Progress has also been made in the production of granules that can be sprayed using conventional spraying equipment. To

date, nine different granular and seven different tablet formulations were developed. In addition, a range of carriers and granulation solvents were screened to study their effect on fungal viability.

During the past twelve months, conditions were optimised for the production of blastospores in stirred, small scale fermentation, tank reactors. Improvements were made in the large scale production of: (1) *B. brongniartii* using a conventional diphasic fermentation step and (2) *M. anisopliae* using a solid state fermentation system. Quality control parameters for both systems were refined to fulfil the requirements and procedures of the **EU directive 91/414** and its Annexes.

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 were refined to optimize detection of *Beauveria* and *Metarhizium* at the species and strain levels. Analyses of the PCR products (rDNA and mtDNA RFLPs) were finalised during this report period. Particular emphasis was placed on RFLPs of the mtDNA because most of the BIPESCO strains could be easily differentiated with this technique. Further specific primers will be designed to check for similarities/differences for all isolates in the BIPESCO collection.

Knowledge of the spatial-temporal distribution of inoculum is essential to our understanding how epizootics are initiated. Studies on pest and fungal ecology and the effect of environmental factors on host-pathogen interactions are ongoing. Field studies were conducted to determine the persistence of *B. brongniartii* and *M. anisopliae* in the soil at sites where these pathogens had been/or are being used extensively for scarab and weevil control. In spring 2000, field trials were conducted at old and new trial sites in Austria, Denmark, Germany, Italy and Switzerland. In addition to existing (i.e. ongoing short and long term) trials, new trials were initiated. Soil samples were taken from treated and untreated (control) plots to monitor the population dynamics of the pathogens. Incorporation of conidia into soils is thought to increase their survival by protecting propagules from solar radiation and buffering them against extreme temperatures and moisture. Studies to date show that encapsulated propagules may perform as well as, if not better than, injected inoculum. Encapsulation may enhance percolation through and persistence of the BCA in the soil. Trials will be initiated to test a range of different formulations to see which ones improve the persistence of propagules in different soil types and habitats within the EU.

To learn more about the host range and potential impact of BCAs on non-target organisms we screened selected BIPESCO strains at different doses against selected non-target invertebrates (i.e. earthworms and beneficial species of Carabidae and Staphylinidae). This information will be invaluable for registration authorities and complies with the requirements of **EU Directive 91/414**.

3. ACHIEVEMENTS

The first two milestones have been achieved by (i) setting up a protocol how to maintain and assay selected pest insects. The BIPESCO-team has established appropriate test methods for conducting assays against target and non-target pests and methods for rearing these organisms

(see also IR-II and III). Eight virulent *Beauveria* and *Metarhizium* strains have been provided to each partner, to be able to compare specific isolates with the already characterised reference strains. Valuable data on morphological and physiological characteristics of the BIPESCO reference strains are now available. (ii) Molecular methods and tools are available for characterising fungal strains. 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 were refined to optimize detection of *Beauveria* and *Metarhizium* at the species and strain levels and are available for the partners.

Registration of a *Beauveria brongniartii* as a BCA in Austria (first effective propagule against *Melolontha* in an EEC-member state). *Beauveria brongniartii* became registered as a biocontrol agent by the Austrian plant protectant legislation (§ 10 PMG; BGBl. I, Nr. 60/1997) with June 2000. In spring 1998, *Beauveria brongniartii*, the successful antagonist against the cockchafer (*Melolontha melolontha*) has been filed by the Austrian company F. Joh. Kwizda GmbH for registration (parent company of GH). The product name is Melocont®-Pilzgerste and is produced by Agrifutur srl. (AGF). The research work (field studies and laboratory work) was all done by LFU and could be finalised with assistance from the EU-FAIR6-CT98-4105 research project. The BCA is also accepted for organic farming on the basis of EU regulation 2092/91.

The first BIPESCO publications are already printed and/or in press in international, refereed scientific journals. The papers provide information that will help in the registration of fungal BCAs. They reflect the highly synergistic interactions between the partners and close co-operation of the BIPESCO team. A reference list can be downloaded from the BIPESCO Homepage <http://bipesco.uibk.ac.at>. The BIPESCO homepage is visited on average by ten individuals/institutions per day.

In February 2000 a conference was organised by BIPESCO on "Biological control of *Melolontha melolontha*" at Auer/Italy. The programme offered "end users" and other participants to discuss with representatives of the BIPESCO team issues pertaining to the use of fungi for the control of the cockchafer (*Melolontha melolontha*). More than 200 farmers, organic growers, research scientists, registration authorities and extension services mainly from Austria, Italy, Germany and Switzerland attended this meeting. Papers presented at the conference were particularly welcomed as submissions to the Laimburg Journal and will be published in the year 2001.

The BIPESCO team is helping to organise the International Symposium on "Bioactive fungal metabolites - impact and exploitation" which is to be held 22-27th April 2001 at the University of Wales, Swansea (UWS). This conference will be of considerable interest to scientists, representatives of industry and government health, food and environmental services and will assess whether metabolites produced by fungal BCAs pose a risk to human and animal health. For more information please see <http://www.biomed.man.ac.uk/bmsconference> or <http://www.swan.ac.uk/conferences/>. The BIPESCO team is also helping organise the "Third Meeting of the *Melolontha* Subgroup IOBC wprs Working Group "Integrated Control of Soil Pests" which is to be held 24th-26th September 2001 in Aosta, Italy. This meeting provides an excellent opportunity for all participants to join with others in a discussion of an important issue concerning the relevance of *Beauveria brongniartii* in a practical context. The

programme will offer the possibility to dialogue on issues of a successful control of the cockchafer (*Melolontha melolontha*). Based on these conferences BIPESCO team-members will prepare two inter-linked demonstration projects.

4. LITERATURE PUBLISHED OR IN PRESS

In the second year BIPESCO-partners have published results in international, refereed, scientific journals, and presented results at international scientific meetings and grower associations.

Abendstein D., Pernfuss B. & Strasser H. (2000). Characterization of *Beauveria brongniartii* by the BIOLOGTM-system. IOBC wprs Bulletin 23 (2), 175-179.

Abendstein, D., Pernfuss, B. & Strasser, H. (2000). Evaluation of *Beauveria brongniartii* and its metabolite oosporein regarding phytotoxicity on seed potatoes. *Biocontrol Science and Technology*, 10: 789-796.

Abendstein, D., Schweigkofler, W. & Strasser, H. (2001). Effect of the fungal metabolite oosporein on feeding behaviour and survival of *Melolontha melolontha*- and *Galleria mellonella*-larvae. Laimburg Journal, accepted.

Abendstein, D., Schweigkofler, W. & Strasser, H. (2001). Effect of the fungal metabolite oosporein on feeding behaviour and survival of *Melolontha melolontha*- and *Galleria mellonella*-larvae. Laimburg Journal, accepted.

Chikwenhere, G. P. & Vestergaard, S. (2001). Pathogenecity and Prevalence of *Beauveria bassiana* (Vuill) Bals. in the Chevron Waterhyacinth Weevil, *Neochetina bruchi* Hustache in laboratory and under field conditions. BioControl, Accepted.

Eilenberg, J., Enkegaard, A., Jensen, D. F. & Vestergaard, S. (1999). Present and future potential for biological control in. International Symposium: Biological Control Agents in Crop and Animal Protection, University of Wales, Swansea, August 24. – 28, 1999.

Eilenberg, J.; Enkegaard, A.; Jensen, D.F. (2000). Present use - and present and future potential - for biological control of pests and diseases on outdoor crops in Denmark. IOBC/WPRS Bulletin, 23, 19-22.

Eilenberg, J.; Enkegaard, A.; Vestergaard, S.; Jensen, B. (2000). Biological control of pest on plant crops in Denmark: Present status and future potential. *Biocontrol Science and Technology*, 10, 703-716.

Enkerli, J., Widmer, F., Gessler, C. & Keller, S. (2001). Strain specific microsatellite markers in the entomopathogenic fungus *Beauveria brongniartii*. *Mycological Research*, submitted.

Inglis, G.D., Goettel, M.S., Butt, T.M. & Strasser, H. (2001). Use of hyphomycetous fungi for managing insect pests. In: *Fungal Biocontrol Agents: Progress, Problems & Potential* (Butt, T.M., Jackson, C., Magan, N., Eds.). CABI Wallingford.

Inglis, G.D., Goettel, M.S., Butt, T.M. & Strasser, H. (2001). Use of hyphomycetous fungi for managing insect pests. In: *Fungal Biocontrol Agents: Progress, Problems & Potential* (Butt, T.M., Jackson, C., Magan, N., Eds.). CABI Wallingford.

Jensen D. B. & Strasser H. (2000). Estimation of virulence by measuring the N-acetylglucosamine in submerged culture of *Beauveria brongniartii* and *Metarhizium anisopliae*. IOBC wprs Bulletin 23 (2), 203-206.

- Jung, K. & Zimmermann, G. (2000). Erfahrungen mit der Entwicklung eines Biopräparates auf der Basis von *Beauveria brongniartii* zur Bekämpfung von Feld- und Waldmaikäfer. Proceedings Deutsche Pflanzenschutztagung, Weihenstephan, Germany, Nr. 170.
- Jung, K. & Zimmermann, G. (2000). First experiences with the development of a myco-insecticide based on *Beauveria brongniartii* against the field and forest cockchafer and related species. 23th Annual Meeting Soc. Invert. Pathol., Guanajuato, Mexico. Abstract p. 61.
- Keller, S. (2000). Erfahrungen in der Engerlingsbekämpfung mit dem Pilz *Beauveria brongniartii*. - Mitt. deutsch. Ges. allg. angew. Ent. 12, 111-114.
- Keller, S. (2000). Use of *Beauveria brongniartii* in Switzerland and its acceptance by farmers. - Bull. IOBC/WPRS 23(8), 67-71.
- Keller, S., David-Henriet, A.-I. & Schweizer, C. (2000). Insect pathogenic soil fungi from *Melolontha melolontha* control sites in the canton Thurgau. - Bull. IOBC/WPRS 23(8), 73-78.
- Keller, S., Forrer, H.R. & Fried, P.M. (2000). Experiences in white grub control with the fungus *Beauveria brongniartii*. - Poster, IFOAM, Basel, 28.-31.9.2000.
- Mavridou, A., Cannone, J. & Typas, M.A. (2000). Identification of group-I introns at three different positions within the 28S rDNA gene of the entomopathogenic fungus *Metarhizium anisopliae* var. *anisopliae*. Fungal Genetics and Biology: in press.
- Pernfuss, B., Abendstein, D., & Strasser, H. (2001). Virulence of Entomopathogenic Fungi - Mini Review. Laimburg Journal, accepted.
- Schweigkofler, W. (2001). Assessment of scarab infestation in Northern Italy. Laimburg Journal, accepted.
- Schweigkofler, W. (2001). Pathogene des Maikäfers - (Pathogens of the cockchafer). Laimburg Journal, accepted.
- Schweigkofler, W. (2001). Untersuchungsmethoden für entomopathogene Pilze unter besonderer Berücksichtigung des Maikäferpathogens *Beauveria brongniartii*. (Methods to evaluate entomopathogenous fungi focusing on the pathogen of cockchafers *Beauveria brongniartii*). Laimburg Journal, accepted.
- Strasser, H. & Abendstein D. (2000). Oosporein, a fungal secondary metabolite with antimicrobial properties. IOBC wprs Bulletin 23 (8), 113-115.
- Strasser, H. (2000). A researcher's obstacle race: report on the registration of a new bioinsecticide. IOBC wprs Bulletin 23 (2), 9-13.
- Strasser, H. (2000). Searching for alternative biological Pest control agents against *Phyllopertha horticola* (L.) IOBC wprs Bulletin 23 (2), 23-27.
- Strasser, H., Pernfuss, B. & Abendstein D. (2000). Zum EU-Projekttreffen zur biologischen Bekämpfung von Maikäfern und anderen Insektenschädlingen. Obstbau & Weinbau, 10: 304.
- Strasser, H. (2001). Assessment of Scarab infestation in Austria. Laimburg Journal, accepted
- Strasser, H. (2001). Biocontrol of important soil dwelling pests by improving the efficacy of insect pathogenic fungi (BIPESCO): Project funded by the European Union - RTD programme FAIR6-CT98-4105. Laimburg Journal, accepted.
- Strasser, H., Abendstein, D., Stuppner, H. & Butt, T. (2000). Monitoring the distribution of secondary metabolites produced by the entomopathogenous fungus *Beauveria brongniartii* with particular reference to oosporein. *Mycological Research*, 10: 1227-1233.

- Strasser, H., Abendstein, D., Stuppner, H. & Butt, T. (2000). Monitoring the distribution of secondary metabolites produced by the entomopathogenic fungus *Beauveria brongniartii* with particular reference to oosporein. *Mycological Research*, 10: 1227-1233.
- Strasser, H., Butt, T.M. & Vey, A. (2000). Are there any risks in using entomopathogenic fungi for pest control, with particular reference to the bioactive metabolites of *Metarhizium*, *Tolypocladium* and *Beauveria* species? *Biocontrol Science and Technology*, 10: 717-735.
- Strasser, H., Vey, A. & Butt, T.M. (2000). Are there any risks in using entomopathogenic fungi for pest control, with particular reference to the bioactive metabolites of *Metarhizium*, *Tolypocladium* and *Beauveria* species? *Biocontrol Science and Technology*, 10: 717-735.
- Vestergaard, S. & Eilenberg, J. (1999). Non-target effects of *Metarhizium anisopliae* on ground and rove beetles (Carabidae and Staphylinidae) after application in a Lucerne field. 32th Annual Meeting, Society for Invertebrate Pathology, Irvine, California, USA, August 23. – 27, 1999.
- Vestergaard, S., Eilenberg, J. & Harding, S. (1999). Microbial control of scarabs and weevils in Christmas tree and decoration green plantations. International Symposium: Biological Control Agents in Crop and Animal Protection, University of Wales, Swansea, August 24. – 28, 1999.
- Vestergaard, S., Frølander, A., Eilenberg, J. & Harding, S. (2000). Biologisk bekæmpelse af oldenborre og snudebiller i juletræer og klippegrønt [Biological control of scarabs and weevils in Christmas tree and decoration green plantations]. Poster presented at Skov- og Lanskabskonferencen, Nyborg. Februar, 2000. (in Danish).
- Vestergaard, S.; Eilenberg, J.; Harding, S. (2000). Potential for microbial control of scarabs and weevils in Danish forestry. *IOBC/WPRS Bulletin*, 23, 35-37.
- Wern, V. (1999). Billerne ud af juletræerne [Christmas trees without beetles]. *Berlingske Tidende* (Major National Newspaper). Section Univers 21. dec. pp. 8-9 (in Danish).
- Zelger, T. & Schweigkofler, W. (2001). Persistence of *Beauveria brongniartii* blastospores and conidia in non-sterile and semi-sterile soils. *Laimburg Journal*, accepted.
- Zimmermann, G. & Jung, K. (2000). Vorkommen von Feld- und Waldmaikäfer sowie Junikäfer und Gartenlaubkäfer in Deutschland und Bekämpfungserfahrungen: Ergebnisse einer Umfrage. *Proceedings Deutsche Pflanzenschutztagung*, Weihenstephan, Germany, Nr. 450.
- Zimmermann, G. & Jung, K. (2001). Auftreten und Bekämpfung von Feld- und Waldmaikäfer in Deutschland: Aktuelle Situation. *Laimburg Journal*, accepted.