Doctoral Thesis

Influence of soil factors on virulence, growth and survival of the fungus Beauveria brongniartii, a specific biocontrol agent of the European cockchafer (Melolontha melolontha)

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INFLUENCE OF SOIL FACTORS ON VIRULENCE, GROWTH AND SURVIVAL OF THE FUNGUS BEAUVERIA BRONGNIARTII, A SPECIFIC BIOCONTROL AGENT OF THE EUROPEAN COCKCHAFAER (MELOLONTHA MELOLONTHA)

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The haploid filamentous hyphomycete fungus *Beauveria brongniartii* (Sacc.) Petch (Deuteromycotina: Hyphomycetes) has been identified as a major pathogen of the European cockchafer *Melolontha melolontha* L. (Coleoptera: Scarabaeidae) more than 100 years ago and its potential as a biological control agent (BCA) has been recognised from early on. The larvae of *M. melolontha* can cause heavy damages in grassland, arboriculture and vineyards. Since 1991, a product based on sterilised barley kernels colonised with *B. brongniartii* has been commercially available in Switzerland. The kernels are tilled into the soil with the aim to produce sufficient fungal material to initiate an epizootic in the host population. The success of such a strategy is strongly dependent on the efficacy and persistence of highly virulent fungal material in the soil environment. Therefore knowledge on virulence, growth and survival of the BCA in the soil are important aspects in the process of developing and improving biological control strategies.

The selection of virulent strains is one prerequisite for an efficient control of insects using fungal pathogens. The virulence of 22 strains of *B. brongniartii* against larvae of *M. melolontha* was investigated. It has been demonstrated that the variability of the efficacy of different strains was considerably high. Bioassays conducted with blastospores revealed better efficacy (expressed as LT50) than bioassays performed with conidia. But the assessment of the relative virulence between different strains was consistent with both spore types. The strains used in the commercial products have been proven to be highly virulent against *M. melolontha*. Furthermore, it has been shown that factors like age and origin of the Melolontha larvae influence the efficacy in the same extend as the virulence and spore types themselves.

Optimal growth and sporulation of *B. brongniartii* on the barley kernels are crucial requirements to introduce enough infective propagules into the soil to initiate a control of the host. The effects of abiotic and biotic soil factors on occurrence of *B. brongniartii* were studied in different regions in Switzerland. It has been demonstrated that soil temperature mainly affected occurrence and density of *B. brongniartii* after application. Optimal temperatures favoured the growth of *B. brongniartii* after application in spring and summer, whereas cold temperatures were responsible for the failure of the application in autumn. Laboratory experiments revealed that the growth rate was reduced at cool temperatures. Therefore the development of the inoculum in the soil after an autumn application has been
slowed down during wintertime and irreversibly stopped. The application in soils with higher clay content resulted in a higher density of *B. brongniartii*. Contrary, increased microbiological soil activity (expressed as catalase activity) negatively influenced the increase of the fungal density after application. Following laboratory experiments confirmed that the increase was different in three native soils (sand, loam and clay), but was not correlated with different clay contents of the soil. In sterilised soil, though, the differences were not detected, suggesting that biotic factors have a greater influence than soil texture.

To demonstrate that the applied BCA strain can be re-isolated from the soil three months after the BCA application, 43 isolates from soil samples were characterised using 6 strain-specific microsatellite markers. This study revealed that the applied BCA can be re-isolated from the plots where it has been applied using the existing isolation methods. We assume that the increase in fungal density after the BCA treatment is a consequence of the BCA application.

The survival of *B. brongniartii* and its efficacy against *M. melolontha* was examined during 16 months after application as a BCA in different soils in Switzerland. In absence of *M. melolontha*, the reduction of the number of colony forming units (CFU) in the soil was nearly 90% during the time monitored. Furthermore it has been shown, that the decline of the CFU density of *B. brongniartii* was more pronounced in soils with high organic content and increased microbiological activity. At sites with high host density, the survival was significantly longer and the fungal density after 16 months was at a sufficient level to exert control in the following host generation. The rapid decrease of the fungus in the absence of the host gives further evidence of the high specificity of the fungus and that a saprophytic multiplication without the host is unlikely.

Finally, the results of this thesis demonstrated that the success of the application of *B. brongniartii* against *M. melolontha* depends on a number of factors. The identification and understanding of these factors are important for optimising the existing application strategy as well as for the development of new BCA formulations based on hyphomycete fungi.