Beauveria spp. infecting *Ips typographus*, *in vitro* isolation and virulence bioassay – preliminary results

Marek Barta[1,2], Ján Ferenčík[1,3,5], Ivona Kautmanová[1,4], Milan Kozánek[1]

[1]Institute of Zoology, Slovak Academy of Sciences, Dúbravská cesta 9, 845 06 Bratislava, email: milan.kozanek@savba.sk, [2]Arboretum Mlynany SAS, Vieska nad Žitavou 178, 951 52 Slepčany, email: marek.barta@savba.sk, [3]State Forests of TANAP, 059 60 Tatranská Lomnica, [4]Natural History Museum Bratislava, Vajanského nábr. 2, 810 06 Bratislava, [5]Scientica s.r.o., Hybešova 33, 831 06 Bratislava, Slovakia

Abstract. During this study 633 adults of *Ips typographus* showing characteristic symptoms of *Beauveria* mycosis were collected in natural populations throughout Slovakia. Eighty-one collecting sites were visited and as many as 316 isolates of *Beauveria* sp. were obtained using a selective agar medium. Altogether 68 *Beauveria* isolates were screened for their virulence against adults of *I. typographus*. These preliminary screening bioassays proved biological activity of the isolates against the test insects, however virulence varied greatly among isolates. Average mortality of test insects ranged between 10.87-95.83% depending upon isolate. The laboratory results indicate the potential of 8 *Beauveria* isolates as a biocontrol agent against *I. typographus*. Those isolates killed >80% of test insects in the bioassays.

Introduction

European spruce bark beetle (ESBB), *Ips typographus* L., belongs to the most dangerous insect pests of spruce forests in Central Europe. It is generally agreed that south-exposed, sunlit, and >60 year-old spruce trees are preferably attacked by ESBB. Spruce trees of poor physiological conditions affected by, for instance, diseases or windstorms are also attractive to ESBB. Disastrous windstorm in High Tatras in November 2004, when over 12 000 ha of forests were damaged, gave rise to an enormous propagation of ESBB in the affected spruce forests. In order to minimize ESBB attacks on living trees traditional measures including clearing windthrows, sanitation felling of infested trees, and installation of trapping devices have been applied. Natural antagonists reducing ESBB populations, mostly fungi, protozoa, nematodes, or hymenopterans, have also been studied to evaluate their regulatory capacity.

Beauveria bassiana (Balsamo) Vuillemin is ubiquitous entomopathogenic fungus that attacks various arthropods by causing acute mycoses. It can spread fast among hosts horizontally via aerially produced conidia and infects its host by penetration of the cuticle by germ hyphae (Hajek and St Leger, 1994). Many strains of *B. bassiana* have been isolated and tested against different pests in various cropping systems. Selected strains have been successfully licensed for commercial use against whiteflies, aphids, thrips, and numerous other insect pests (Shah and Pell, 2003). *B. bassiana* is also regularly observed in natural populations of ESBB (Landa et al., 2001; Michalková et al., 2012), however it has not yet been commercially applied against ESBB populations.

The objective of this study was (1) to isolate native strains of *Beauveria* spp. from naturally infected ESBB adults, (2) to propagate the strains in vitro conditions, and (3) to design and optimize a laboratory bioassay for virulence evaluation.

Data and methods

In vitro isolation and cultivation of *Beauveria* strains:

In vitro isolates were obtained by incubating mycosed ESBB adults collected from nature on selective agar medium (Sabouraud-dextrose agar (SDA) suppl. with 0.2g/l dodine, 0.6g/l streptomycin, 0.05g/l tetracycline and 0.25g/l cyclohexadine). Fungal colonies growing on the selective medium were subsequently subcultured on standard SDA medium in Petri dishes at 25±2°C. The cultures on SDA were used for virulence bioassay.

Virulence bioassay:

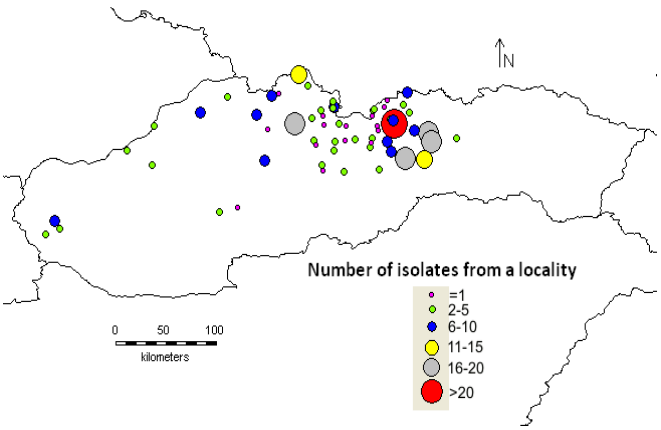
Conidia harvested from 21-day-old *in vitro* cultures were suspended in 100 ml of 0.05% (w/v) water solution of Tween 80. Conidia concentration in the suspension was adjusted to 1×10^6 conidia/ml for all isolates. Virulence of *Beauveria* isolates was determined by average mortality of test ESBB adults after their treatment with the conidia suspensions. A group of 150 ESBB adults (50 individuals in 3 repetitions) were treated with the conidia suspension by their dipping into the suspension for 3 s. An additional group of 100 ESBB was used as a control. The control insects were treated with sterilised 0.05% water solution of Tween 80 (w/v). All treated insects were maintained individually in Petri dishes at 25±2°C and natural photoperiod for 10 days. They were watched daily to monitor *Beauveria* infection.

Results and discussion

During the survey 633 ESBB adults with typical symptoms of *Beauveria* infection were collected from 81 localities (collecting sites) throughout Slovakia. Out of the mycosed individuals 316 isolates were obtained using the selective agar medium. The isolates come from altogether 69 collecting sites. Localities, from where *Beauveria* isolates were successfully obtained, are shown on a map (Fig. 1). The localities are distinguished by a total number of obtained isolates. In the greatest number of localities only one isolate was obtained (19 localities). Two isolates

were obtained from 13 localities. In 37 localities more than three isolates were obtained and in seven localities ≥10 isolates were isolated. As many as 25 isolates were obtained from one locality. It was the greatest number of isolates per a single locality. As shown on Fig. 1 collecting sites are mostly situated in the northern part of the country. However, western (e.g. Malé Karpaty, Biele Karpaty, Kysuce) and central (e.g. Štiavnické vrchy, Poľana) regions are also included. All isolates were subjected to microscopic evaluation to confirm their taxonomic status within *Beauveria* genus. Over 100 isolates have been selected on the basis of the microscopic observations and are being analyzed for species diversity by DNA sequencing.

Fig.1 Localities in Slovakia where *Beauveria* spp. strains were isolated

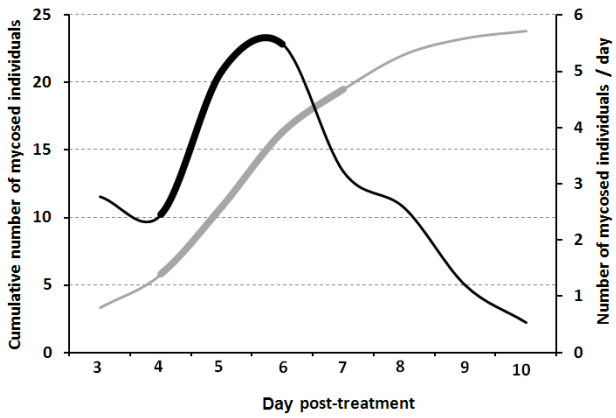


Up to now a pathogenicity of 68 isolates has been tested against ESBB adults. The virulence bioassays revealed a high variability among tested isolates and average mortality of ESBB induced by the *Beauveria* isolates ranged between 10.87-95.83%. Average mortality of ESBB below 50% was recorded for 28 isolates. Altogether 32 isolates brought about 51-80% mortality of test insects. Only 5 and 3 isolates killed 81-90% and >90% of test individuals, respectively. Dynamics in mortality rate of treated ESBB adults (no. of killed individ./24h) is presented in Fig. 2. Monitoring of mortality in the virulence bioassay started on 3rd day after treatment and continued till 10th day. On average, mortality rate increased till 6th day, when it culminated. At the culmination the mortality rate reached 5.49 dead adults/24h. After this point the mortality rate continuously decreased till 10th day, when average number of killed ESBB adults decreased to 0.53 individ./24h. The greatest average mortality rate (approx. 5 killed individ./24h) was recorded on 5th and 6th day. Average time to death for all tested isolates reached 5.83 days. This time was shorter (5.28 days) for the eight isolates demonstrating higher virulence (>80% mortality). As it can be seen in Fig. 2 mortality rate on 3rd day was higher than that on 4th day. This was due probably to the fact that the mortality monitoring started only on 3rd day after treatment, but first killed insects could appear earlier (on 2nd day). And these were recorded only together with those died on 3rd day, what finally led to increased mortality rate on this day.

Conclusion

1. *Beauveria* mycoses were common in natural populations of *I. typographus* in Slovakia, however no epizootics were observed.
2. The selective medium used in this study was suitable for isolation of *Beauveria* strains from mycosed ESBB adults.
3. In preliminary screening bioassay the tested *Beauveria* isolates proved biological activity against adults of *I. typographus*, however virulence varied greatly among isolates. The most virulent isolates brought about >90% mortality in populations of test individuals.
4. The laboratory results indicate the potential of 8 *Beauveria* isolates as a biocontrol agent against *I. typographus*. Those isolates killed >80% of test insects in bioassays.

Fig.2 Average mortality dynamics of treated adults during the virulence bioassay; ▬ mortality rate (dead individ./24h), ▬ cumulative mortality



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