

Treatment of bark beetle attacked trees with entomopathogenic fungus *Beauveria bassiana* (Balsamo) Vuillemin

Rastislav Jakuš ✉ and Miroslav Blaženec

Slovak Academy of Sciences, Institute of Forest Ecology, Štúrova 2, 960 53 Zvolen, Slovak Republic, phone: +421 905 217 169, fax: +421 45 5334686, e-mail: jakus@savzv.sk

ABSTRACT

We carried out an experiment with using the entomopathogenic fungus *Beauveria bassiana* (Bals.) Vuill. for sanitation of active infested trees. We used 15 active infested trees from which 5 stems were treated with an insecticide, 5 were treated with solution of the tested entomopathogenic fungus and 5 were left as control. The used insecticide was pyretroid Fury 10 EW. We used a biopreparation based on the entomopathogenic fungus *B. bassiana* in form of wettable powder. The material was diluted. The suspension contained 10^7 spores of the tested entomopathogenic fungus in 1 ml. The suspension was applied across the whole stem surface. We succeeded to infect about 28.75% of *Ips typographus* L. individuals in the treated stems. The number of live individuals was comparable with the variant using the insecticide.

KEY WORDS

Ips typographus, *Beauveria bassiana*, Norway spruce, attacked trees

INTRODUCTION

Entomopathogenic fungus *Beauveria bassiana* (Balsamo) Vuillemin grows naturally in all continents, except Antarctic. The fungus has been isolated from more than 700 insect species belonging to nine orders. The most hosts have been identified among Lepidoptera and Coleoptera. *B. bassiana* has a potential to be used as a biological insecticide against more than 70 insect species (Aleshina 1980). Its effect has been tested both in the laboratory and in field for a large number of pests as thrips, whiteflies and aphids. It is able to infect all immature stages and adults of bark beetles and in suitable

conditions kill them. This insect pathogen naturally occur in *Ips typographus* (L.) populations (Wegensteiner, Weiser, Führer 1996; Landa *et al.* 2001).

The fungus creates a milky whitish mycelium on artificial substrates as well as on natural hosts. The most frequent ways of *B. bassiana* penetration into the host are through the cuticle and stigmata. Apart from this, the infection can also penetrate *per os*, especially in case of species with biting mouth parts; and there have been recorded also infections through respiratory system and mouth. Insects are infected with conidia, first germinating on the cuticle surface and, after a short period of growing on the surface, penetrating their fila-

ments orthogonally the chitin surface of the cuticle and continuing inside the body cavity. Growing germination filaments decomposes the surface by releasing enzymes. At the moment of reaching the host's body cavity, the fungus starts to produce a secondary metabolite beauvericin debilitating the immune system of the host. After the death of the host, the fungus produces an antibiotic (oosporein) enabling the fungus to compete with intestinal bacteria. Inside the cadaver are formed cylindrical conidia, endoconidia (blastospores), commonly $2-3 \times 7 \mu\text{m}$ in size. The conidia on fructifying filaments occur in various numbers – by two or more on short stalks. The endoconidia produce other hyphae on which somewhat later, new endoconidia are created. The growing hyphae fill the insect body (Weiser 1966), and at a sufficient moisture content (92% and more) they reach the body surface. The surface of the mummified cadaver is progressively grown with elevating filaments with developing aerial conidia. Their growth is optimal at a temperature 23–26°C, and a relative air humidity or substrate moisture content of 80–100%. Minimum temperature for mycelium growth is 5–8°C, maximum 28–31°C. In nature, *B. bassiana* persists in surface soil layers in form of mycelium in host cadavers on one hand, and on the other hand in organic residua (saprophyte phase). In spite of a very large number of hosts, epizootic phenomena in natural populations of pests caused by the fungus *B. bassiana* were investigated only scarcely (Landa 2008).

Occurrence of the fungus *B. bassiana* on dead hosts is distinctly recognisable in case when the fungus has created a well-developed culture on the cadaver surface. *B. bassiana* creates a short, dense mycelium, and sporulation is accompanied by touch-of-dust-resembling structures formed on the mycelium surface. The fungus begins with puncturing joints between the individual body segments or parts (interface between prothorax and mesothorax, soft tissues on abdomen, joints between the individual segments of legs and wings) and entering the natural body openings (mouthparts). Mycelium growing in these areas is the chief symptom of the direct link between the host and pathogen. In a similar way, it is possible to observe mycelium on body surface of all larval instars and on pupae (Landa 2008).

B. bassiana is a typical representative of entomopathogenic soil mycoflora. The fungus is distributed throughout the entire world. In spite of the fact that this

entomopathogenic fungus is typical for the soil mycoflora, its utilisation in commercial preparations has been focussed on pests feeding on leaves. Bio-preparations based of this fungus are registered for application against several insect species (Landa *et al.* 2007).

Entomopathogenic fungi are applied either as spores suspended in water or as spores formulated and applied as powder. Terrestrial or aerial applications are possible. *B. bassiana* can either be applied in form similar to pesticides or special pheromone traps can be used to infect the bark beetle population (Vaupel and Zimmermann 1996; Kreutz, Vaupel and Zimmermann 2004; Landa *et al.* 2007; Kunca *et al.* 2009; Vakula *et al.* 2010). Kreutz, Zimmermann and Vaupel (2004) have shown the horizontal transmission of *B. bassiana* between adults of *I. typographus* in laboratory and field conditions. Application of this fungus in field can cause 100% mortality of bark beetles (Battay 2007). However, successful infection is strongly dependent on appropriate environmental conditions, with sufficient moisture often being critical (Krischik and Davidson 2004). In general *B. bassiana* is characterised as having no harmful effects on a number of non-target organisms. *B. bassiana* is not fully selective to herbivorous insects. The effect on predators of bark beetles, however, is minimal in comparison to mortality caused to bark beetles (Steinwender and Wegensteiner 2008). The fungus is mostly applied in form of spraying with conidia suspension. The biopreparations represents possible alternative to the use of insecticides in forest protection.

Biosubstances (biopreparates) on the base of *B. bassiana* can be in general characterised as contact substances, which means that it is absolutely necessary to ensure the contact between the substance (spores) and the target pest species.

The aim of work was to test the application of bio-substance based on *B. bassiana* for sanitation of active bark beetle attacked tree.

METHODS

Experiment

In July 2007 we carried out an experiment with using the entomopathogenic fungus *B. bassiana* for sanitation of active infested trees. The experimental stands were situated in the area of the Spišská Magura Mts.,

near the village of Slovenská Ves (community forest Výborná) in declining spruce stands (1000 m asl) with a particularly high population of bark beetles. We used 15 cut active attacked trees from which 5 stems were treated with an insecticide, 5 were treated with solution of the tested entomopathogenic fungus and 5 were left as control. The used insecticide was pyrethroid Fury 10 EW (active Ingredient: zeta-cypermethrin 10%) applied following the standard directions for use. We have used water solution of insecticide in concentration 0.7%. The liquid consumption for spraying of 1 m³ wood volume was 5–6 l.

Biosubstance with the entomopathogenic fungus and its application

In our experiments we used a biopreparation based on the entomopathogenic fungus *B. bassiana* in form of wettable powder (WP) – spores finalised with an inert carrier, at a minimum concentration of 1.0×10^{10} spores/1 g, light whitish powder, precise doses (± 0.1 g) in plastic containers – each for a single use. The fungal preparation was diluted in water: 1g biopreparation/1000 ml water with 3 drops of a common detergent (Jar) as a wetting agent. The suspension contained 1.0×10^7 spores of *B. bassiana* in 1 ml. The suspension was applied with an insecticide backpack sprayer directly on the bark surface, across the whole stem surface.

To provide effective treatment over the whole trunk surface ensuring that the suspense would penetrate spontaneously after the application, rather high volumes of the suspension were necessary to apply. For this type of application, medicinal herb terminology uses the term „run off“ indicating that the substance is applied in amounts sufficient to remove from the stem in form of stem flow. The average suspension consumption for spraying of 1 m³ wood volume was approximately 6 l.

Evaluation of the experiment

After the end of the growing season (in October 2007), bark beetles were sampled together with bark. One sample, 20 × 50 cm in size, was taken from the central part of each trunk. Individual beetles left on the bark surface after the sampling were collected too. The samples were placed in plastic bags, stored in a refrigerator, and then evaluated in the laboratory.

Macroscopic survey

The sampled material was evaluated with the aid of a stereoscopic microscope. Bigger bark pieces were cut in strips easy to move across the range of view of the binocular microscope. The direct evaluation after the sampling divided the samples into the following categories:

- a) live = examining the adults of *I. typographus* we recorded motions;
- b) immobile = no motion was recorded; and concerning the sampling date, significant numbers of already diapausing adults of *I. typographus* can be supposed in this category;
- c) infected = distinct mycelium growth recorded on body surface of *I. typographus* adults.

Verification of causal agents of diseases recorded at samples evaluation

For incubation we used sterile wet cells (plastic Petri dishes with a 2%-agar layer or a wet sterile filtering paper on the bottom). The individuals designed for analyses were placed on the agar or filtering-paper surface, the wet cells were closed and placed in an environment favourable for incubation ($23 \pm 1^\circ\text{C}$). The incubation took 2–4 days. This time and conditions allow the fungus *B. bassiana* to run its complete developmental cycle. The purpose of incubation was to promote the growth of *B. bassiana* on body surface of infected individuals and to reach as soon as possible the final phase of the developmental cycle of the pathogen – sporulation on the aerial mycelium. In case that it was not possible to identify the aetiology macroscopically, the culture was excluded from the further processing (= cultivation, clearing, identification).

Statistical processing

Assumptions for use of parametric statistics were tested (Shapiro-Wilk test and Levene test; Underwood 2001). The data did not comply with the assumptions, the non-parametric Kruskal-Wallis test and then Mann-Whitney U test were used. All statistical calculations were done using Statistica 9 software.

RESULTS

The descriptive statistics from obtained data are shown in Tab. 1. The percentage of *I. typographus* individuals infected with entomopathogenic fungus *B. bassiana* on trunks treated with biopreparation was significantly higher than in the case of control (K-W test: $H(2, N = 15) = 9.7123$, $p = 0.0078$; M-W test: $Z = -2.6111$, $p = 0.0079$) or trunks treated with insecticide (M-W test: $Z = -2.6111$, $p = 0.0079$). No statistical differences were found between control and trunks treated with insecticide (M-W test: $Z = -0.8356$, $p = 0.4034$) (Fig. 1).

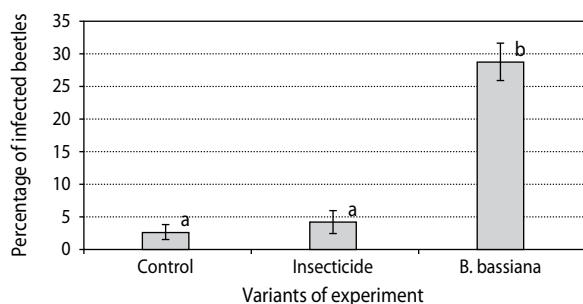


Fig. 1. Mean percentages and SEs of *Ips typographus* individuals infected with entomopathogenic fungus *Beauveria bassiana* in experiment comparing efficiency of two different treatments applied on active attacked trees. Bars with the same letter are not different according to the Mann-Whitney U test

In case of active infested trees we succeeded to infect about 28.75% of *I. typographus* individuals in the

treated trunks. There were no statistical differences in number of alive individuals between trunks treated with biopreparation and trunks treated with insecticide (K-W test: $H(2, N = 15) = 5.4447$, $p = 0.0657$; M-W test: $Z = -0.2089$, $p = 0.8345$). The number of alive individuals on control was significantly higher than on both treated variants (M-W test “control vs. biopreparation”: $Z = 1.9845$, $p = 0.0472$; M-W test “control vs. insecticide”: $Z = 1.9845$, $p = 0.0472$) (Fig. 2).

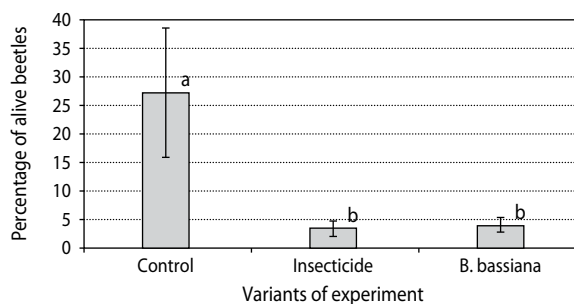


Fig. 2. Mean percentages and SEs of alive *Ips typographus* individuals in experiment comparing efficiency of different treatments applied on active attacked trees. Bars with the same letter are not different according to the Mann-Whitney U test

Except one, all the samples taken from the trunks treated with *B. bassiana*, manifested occurrence of the pathogen in direct association with adults of *I. typographus* at frequencies higher than in the other two variants. The entomopathogenic fungus *B. bassiana* was also present in samples taken from untreated trees or the trees treated with insecticide alone. This fact demonstrates natural occurrence of the native strain of this

Tab. 1. Basic statistical description of samples categories from particular variants of experiment

Variants of experiment	Sample category	Valid N	Mean	Min.	Max.	Std. Dev	Std. Error
control	live	5	13.200	2	36	13.700	6.127
	immobile	5	36.800	15	65	21.253	9.505
	infected	5	1.200	0	2	0.837	0.374
insecticide	live	5	1.600	0	4	1.517	0.678
	immobile	5	39.200	9	76	25.074	11.213
	infected	5	2.600	0	9	3.647	1.631
B. bassiana	live	5	13.200	2	36	13.700	6.127
	immobile	5	36.800	15	65	21.253	9.505
	infected	5	1.200	0	2	0.837	0.374

pathogen (the fungus evidently adapts to the local environment). In the studied locality we also recorded occurrence of two other fungal species growing on body surface of adult beetles. Nevertheless, the determination of other species was not the subject of this study.

DISCUSSION

B. bassiana (and entomopathogenic fungi in general) acts linked also with a phenomenon of mortality that cannot be attributed to this pathogen, but, on the other hand, it lacks presence of an evident symptom pointing at a specific causal agent of the fungus-related mortality. This phenomenon is most frequently the result of unfavourable conditions (temperature, relative air humidity...) hindering the pathogen to develop as saprophyte on host cadavers. These aspects must be considered in evaluation of results.

In laboratory bioassays, the mortality of *I. typographus* caused by *B. bassiana* could reach 88–100% (Vaupel and Zimmermann 1996; Kreutz *et al.* 2004; Kunca *et al.* 2009). In the conditions of high humidity, the mortality strongly depends on the temperature and the *B. bassiana* dosage (Wegensteiner 1992).

Kreutz (2001) have applied bio-preparation of *B. bassiana* and insecticide (Fastac Forst) on spruce logs just after finishing of their colonisation by *I. typographus*. He has achieved 100% mortality in the case of insecticide and 93% mortality in case of biopreparate. In our experiment, the lower mortality caused both by insecticide and by biopreparation was possibly caused by later stage of bark beetle development in our experiment. The length of bark beetle galleries was longer. Beetles were located in larger distance from entrance holes, thus the probability of direct bark beetle contact with insecticide or biopreparation were lower. In earlier experiment, Vaupel and Zimmermann (1996) achieved 23–29% decrease of *I. typographus* breeding efficiency after application of *B. bassiana* powder. Their results are more similar to mortality achieved in our experiment. The timing of biopreparation application is probably the critical factor. We have achieved similar level of mortality (percentage of alive beetles) by application of *B. bassiana* as with application of insecticide. Kreutz (2001) had similar results. It shows that the effectiveness of used biopreparation are comparable with the use of

insecticide. Further work on this method of forest protection should be focused to optimisation of the application timing.

CONCLUSIONS

The results of our experiments carried out on active infested trees manifest a potential of this method in forest protection. The use of biopreparation based on *B. bassiana* could be as effective as the use of insecticides.

ACKNOWLEDGEMENT

This publication is the result of the project implementation: Centrum of excellence of biological methods of forest protection (ITMS: 26220120008), supported by the Research & Development Operational Programme funded by the ERDF. Authors with to thanks to Professor Zdeňek Landa (University of South Bohemia in České Budějovice) and employees of community forest Výborná for help with experiment.

REFERENCES

- Aleshina O.A. 1980. Composition and prospects for study of the entomopathogenic fungi of the USSR. *The Review of Applied Entomology*, 68, 83–84.
- Battay A. 2007. Biocontrol of almond bark beetle (*Scolytus amygdali* Geurin-Meneville, *Coleoptera: Scolytidae*) using *Beauveria bassiana* (Bals.) Vuill. (*Deuteromycotina: Hyphomycetes*). *Journal of Applied Microbiology*, 103 (5), 1406–1414.
- Kreutz J. 2001. Möglichkeiten einer biologischen Bekämpfung des Buchdruckers, *Ips typographus* L. (*Col., Scolytidae*), mit insekten-pathogenen Pilzen in Kombination mit Pheromonfallen. Dissertation zur Erlangung des Grades des Doktors der Naturwissenschaften der Naturwissenschaftlich-technischen Fakultät III -Chemie, Pharmazie und Werkstoffwissenschaften der Universität des Saarlandes, pp. 188.
- Kreutz J., Vaupel O., Zimmermann G. 2004. Efficacy of *Beauveria bassiana* (Bals.) Vuill. against the spruce bark beetle, *Ips typographus* L., in the labo-

- ratory under various conditions. *Journal of Applied Entomology*, 128 (6), 384–389.
- Kreutz J., Zimmermann G., Vaupel O. 2004. Horizontal transmission of the entomopathogenic fungus *Beauveria bassiana* among the spruce bark beetle, *Ips typographus* (Col., Scolytidae) in the laboratory and under field conditions. *Biocontrol Science and Technology*, 14 (8), 837–848.
- Krischik V., Davidson J. 2004. IPM of midwest landscapes. Minnesota Agricultural Experiment Station, pub. number SB-07645, pp. 315.
- Kunca A., Vakula J., Galko J., Gubka A. 2009. Výskum využitia entomopatogénnej huby *Beauveria bassiana* pri ochrane smrečín pred podkôrnými druhmi hmyzu. In: Zborník referátov z medzinárodnej konferencie konanej 8. – 19. 11. 2009 v Nitre "Tretie rastlinolekárské dni Slovenskej rastlinolekárskej spoločnosti" (eds.: K. Hudec, T. Roháčik). Nitra: Slovenská rastlinolekárska spoločnosť, ISBN 978-80-970236-5-2, 95–99.
- Landa Z. 2008. Regulace četnosti populací lýkožrouta smrkového *Ips typographus* pomocí entomopatogenní houby *Beauveria bassiana*. – Metodický postup., Jihočeská univerzita v Českých Budějovicích, Zemědělská fakulta, Katedra rostlinné výroby, Sekce rostlinolékařství, pp. 31.
- Landa Z., Horňák P., Osborne L.S., Nováková A., Bursovová E. 2001a. Entomogenous fungi associated with spruce bark beetle *Ips typographus* L. (Coleoptera, Scolytidae) in the Bohemian Forest. *Silva Gabreta*, 6, 259–272.
- Landa Z., Kalista M., Křenová Z., Vojtěch O. 2008. Praktické použití entomopatogenní houby *Beauveria bassiana* proti lýkožroutu smrkovému *Ips typographus*. In: Ekologické metody ochrany lesa před podkorním hmyzem (eds.: O. Vojtěch, P. Šustr). Sborník referátu ze semináře, Sborníky z výskumu na Šumave – sešit 1. Správa NP a CHKO Šumava Vimperk, 62–71.
- Landa Z., Kalista M., Křenová Z., Vojtěch O. 2007. Využití houby *Beauveria bassiana* v ochraně proti lýkožroutu smrkovému. *Lesnická práce*, 10, 14–15.
- Steinwender B.M., Wegensteiner R. 2008. Effects of *Beauveria bassiana* on the bark beetle *Ips sexdentatus* and on its predator *Thanasimus formicarius*. 41st Annual Meeting of the Society for Invertebrate Pathology and 9th International Conference on *Bacillus thuringiensis*. August 3–7, 2008 University of Warwick, Coventry, United Kingdom.
- Underwood A.J. 2001. Experiments in ecology. Their logical design and interpretation using analysis of variance. Cambridge University Press, pp. 500.
- Vakula J., Varkonda Š., Galko J., Gubka A., Kunca A., Zubřík M. 2010. Rozvoj súčasných technických možností pri štúdiu niektorých spôsobov aplikácie entomopatogénnej huby *Beauveria bassiana* v rámci biologických metód ochrany lesa. In: Aktuálne problémy v ochrane lesa (ed.: A. Kunca). Národné lesnícke centrum, 63–67.
- Vaupel O., Zimmermann G. 1996. Orientierende Versuche zur Kombination von Pheromonfallen mit dem insektenpathogenen Pilz *B. bassiana* (Bals.) Vuill. gegen die Borkenkäferart *Ips typographus* L. (Col., Scolytidae). *Anzeiger Schädlingskunde*, 69, 175–179.
- Wegensteiner R. 1992. Untersuchungen zur Wirkung von *Beauveria* Arten auf *Ips typographus* (Col., Scolytidae). *Mitteilungen der Deutschen Gesellschaft für Allgemeine und Angewandte Entomologie*, 8, 104–106.
- Wegensteiner R., Weiser J., Führer E. 1996. Observations on the occurrence of pathogens in the bark beetle *Ips typographus* L. (Coleoptera, Scolytidae). *Journal of Applied Entomology*, 120, 199–204.
- Weiser J. 1966. Nemoci hmyzu. Nakladatelství ČS akademie věd, Praha, pp. 523.