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The role of fungus *Beauveria bassiana* in reducing the number of *Pissodes castaneus* (Col., Curculionidae) in young forests

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Abstract

Small banded pine weevil *Pissodes castaneus* is one of the most dangerous pests of *Pinus sylvestris* plantations and thickets. The lack of effective and environmentally safe methods of limiting the number of the pest justified to undertake the studies aimed at the laboratory and field evaluation of biological activity of entomopathogenic fungus *Beauveria bassiana* used to reduce the numbers of small banded pine weevil.

In laboratory, the beetles were reared on the sections of fresh Scots pine twigs that were treated with five suspensions containing from 1×10^4 to 1×10^8 conidia of *B. bassiana* in 1 ml. During the 3-week rearing, insect mortality was determined and median lethal concentration LC_{50} was calculated. The field treatments consisted of spraying 4-year-old *P. sylvestris* trees with two formulations of fungus containing 1×10^8 conidia ml^{-1} of suspension. Treatments consisted of spraying 4-year-old *P. sylvestris* trees with two fungus formulations containing 1×10^8 conidia ml^{-1}.

High insecticidal activity of *B. bassiana* was found because the pathogen caused the death of 14–94% of *P. castaneus* beetles, $LC_{50} = 6.51 \times 10^5$ conidia ml^{-1}. Field treatments did not result in the reduction of plant damage caused by small banded pine weevil; therefore, the spraying of trees with *B. bassiana* cannot be recommended to protect the young stands of *P. sylvestris* against pest.

Key words

Beauveria bassiana, *Pissodes castaneus*, *Pinus sylvestris*, biological control

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Introduction

In Poland, protection of young stands against harmful insects is one of the current problems in forestry. It includes the protection of restock areas against small banded pine weevil *Pissodes castaneus* De Geer., which prefers single-species plantations and thickets of Scots pine (*Pinus sylvestris* L.) weakened by biotic factors, mainly pathogenic fungi and deer, as well as abiotic factors including abnormally developed root system, drought, hail and fire.

Small banded pine weevil commonly occurs in Europe, the Asian part of Russia, Turkey and North Africa and, in 2001, was dragged to South America (Panzavolta and Tiberi 2010; Lede *et al*. 2010; CABI 2011). In Poland, it occurs within Scots pine (Kapuściński 1950). The beetles leave the wintering places in the first half of April and they start to feed mainly on buds and last year's young shoots of pines, which with their mass occurrence can lead to a weakening of trees. Most often in May, the females lay eggs around the root collar and on lower parts of pine stems. For the trees, the larvae are more harmful than the beetles because they feed the tunnels under the bark, causing dieback of infested trees (Panzavolta and Tiberi 2010; Kapuściński 1950). In Europe, the species have not been causing an economic problem until the beginning of current century, when the area of its occurrence in Europe increased, including Poland to nearly 8,000 ha per year, leading to the destruction of affected crops (Panzavolta and Tiberi 2010; Brodziak 2011; Jabłoński *et al*. 2016).

In the available literature, there is a little information on how to prevent damage caused by *P. castaneus* in young stands. The only one method used in practice to reduce the population of this species is based on removing and destroying the trees inhabited by weevil. The lack of insecticides registered for pest control eliminates the use of chemical methods during mass occurrence of *P. castaneus* in young stands.

In Poland, for the past 50 years, the small banded pine weevil has not been studying because they were not dangerous from economical point of view. The lack of effective and, at the same time, environmentally safe methods was the reason for taking the studies pointed at the reducing the number of pests in forest plantations and thickets with the use of entomopathogenic fungus *Beauveria bassiana* (Bals.-Criv.) Vuill. The scope of work included laboratory assessment of biological activity of *B. bassiana* and field treatments determining the possibility of using the pathogen to limit the number of *P. castaneus* in restock areas.

Material and methods

Laboratory assessment of biological activity of *B. bassiana*

The beetles used in bioassay were collected in April 2011 from the 3- to 5-year-old Scots pine plantations located in south-east Poland (Forest District Mielec: 50°21′48.8″N 21°36′40.4″E). Following the transfer to the laboratory, the beetles were placed in glass containers and kept fasting for 24 h.

The isolate of *B. bassiana* (original collection number WG, isolated from a soil in South part of Poland, Forestry District Wegierska Górka: 49°36′30.0″N, 19°07′00.1″E) was derived from the collection of the Department of Biology and Environmental Sciences of Cardinal Stefan Wyszynski University in Warsaw, Poland. The conidia was obtained from the fungus culture on agar slants. Next they were inoculated on solid media in culture flasks Roux. After 14 days, the conidia were collected and suspended in 0.02% Triton X-100. Five concentrations of *B. bassiana* containing 1×10^4, 1×10^5, 1×10^6, 1×10^7, 1×10^8 conidia in 1 ml were prepared by serial dilutions and the concentrations of conidia were determined in Thoma cell counting chamber.

The *P. castaneus* beetles were reared on food treated with the suspension of *B. bassiana* conidia. One section of fresh pine twig with a length of 2 cm and a diameter of about 1 cm and one beetle were placed in each sterile Petri dish (10 cm diameter) with filter paper on the bottom. A volume of 200 µl of conidia suspensions was delivered with micropipette to inoculate the bark of twig. Rearing of beetles on the twig sections treated with 200 µl of 0.02% Triton X-100 was a comparative variant of experiment and control-untreated twig sections were treated with 200 µl of distilled water. After 24 h, in all variants, the treated twigs were replaced with untreated ones. In the next days of experiment, the twig sections were replaced for every 72 h.

Altogether seven variants of experiments were established (five suspensions of fungus, comparative and control-untreated variants); in each, 40 beetles were

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tested, a total of 280 insects. The experiment was conducted during 21 days at 22˚C, 70% RH and a photoperiod of 16:8 (L:D) h. Insect mortality was estimated each day, and based on the results, LC_{50} and LC_{90} (concentration required to cause, respectively, 50% and 90% mortality) was calculated using probit regression analysis of fungus concentrations that have been logarithmically transformed according to the Finney method (1962) using POLO PC program, version 2.0 (LeOra Software, USA).

In the case of dead individuals in control-untreated variant, the beetle mortality was calculated using Abbott formula, taking into account the natural mortality of the tested insects. Dead beetles were individually placed in a sterile Petri dishes (diameter 6 cm) with moistened filter paper, and after 48–36 h, they were diagnosed to document mycelium of *B. bassiana* on the basis of morphological characteristic.

Field spraying with *B. bassiana*

Field treatments were performed in 2012 in central Poland (Forest District Celestynów: 52°04′50.6″N; 21°20′54.0″E) in 4-year-old Scots pine plantations with an area of 3 ha in which the small banded pine weevil beetles were feeding on trees in April of the same year. In experimental plantations, more than 90% of trees were weakened by fungi of the genus *Lophodermium*. The treatment was performed in the second half of April, during the period of beetle feeding and egg laying by females on the lower parts of tree stems.

In the experiment 2, isolates of *B. bassiana* were used: the first one tested in laboratory bioassay and the second – comparative isolate – formulated as biopreparation BoVeril (producer Bioved 2005 Kft, Slovakia) in the form of powder containing 5×10^8 conidia g^{-1} (isolate original collection number BB1). Both isolates were used in the form of water suspensions containing 1×10^8 conidia ml^{-1}. The suspensions were prepared 24 h before treatment and stored at 5^0C till the application.

The treatments consisted of spraying the parts of the stems from the root collars to a height of about 20 cm, where the females lay eggs and the larvae hatch. The suspensions were applied with the use of garden sprayer, at 3.5 Ba and a dose of approximately 100 ml of preparation per tree. The comparative control consisted of trees sprayed with the same dose of water. In each variant of the experiment (two suspensions of fungus and untreated), 100 trees were sprayed in the spatial distribution of 20 trees in 5 rows (repetitions).

The efficacy of treatments was estimated after 6 months (the second half of September) based on the numbers of trees with symptoms of colonisation by *P. castaneus*: yellow needles, upper shoots hanging down and resin leaks on the stems. These trees were taken out and stripped in the laboratory, and the larvae located on the stems from the root collar to a height of 20 cm were counted. The numbers of seedlings colonised by the pest as well as the numbers of its larvae in all variants of experiment were compared with the Kruskal–Wallis non-parametric analysis of variance. Differences were considered significant in the post-hoc test when the probability value was lower than the adopted significance level $p \leq 0.05$. Calculations were analysed using Statistica v.10 (StatSoft® Inc. USA) package.

Results

Biological activity of *B. bassiana* in laboratory conditions

Within 3 weeks, a systematic increase in mortality of beetles feeding on twigs treated with *B. bassiana* was observed (Fig. 1). This increase was directly proportional to the concentration of conidia and was the highest in the group of beetles treated with concentrations of 1×10^7 to 1×10^8 conidia ml^{-1}. The mortality increased to more than 50% and 80% of insects after 2 and 3 weeks, respectively.

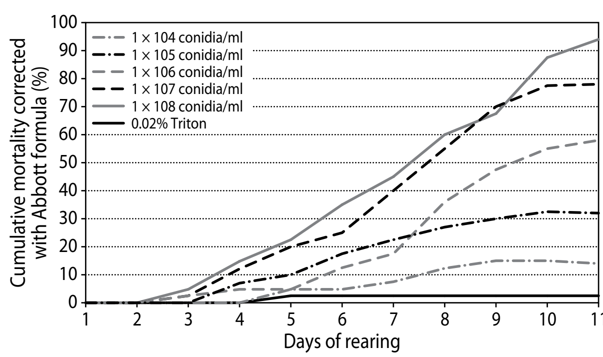

Figure 1. Mortality of *Pissodes castaneus* beetles reared on *Pinus sylvestris* branches treated with *Beauveria bassiana*

After the first week, the mortality of beetles feeding on twigs treated with lower concentrations of the pathogen (1×10^4 to 1×10^6 conidia ml^{-1}) did not exceed 5%. More dynamic increase in insect mortality in these variants was stated in the following weeks: up to 12–36% after 14 days and up to 15–58% in the last day of the experiment.

Mortality of insects on pine twigs treated with 0.02% Triton and untreated ones increased up to 2% in the second week and remained constant to the end of the experiment.

The mortality of insects having contact with the twigs inoculated with fungus was caused by *B. bassiana*, whilst the cause of death of insects in comparative and control variants was unknown.

The results allowed to calculate the lethal concentration of conidia causing 50% (LC_{50}) and 90% (LC_{90}) mortality of tested beetles (Tab. 1).

Table 1. LC_{50} and LC_{90} (conidia ml^{-1}) for *Beauveria bassiana* used against *Pissodes castaneus*

LC_{50}	6.51×10^5
95% limits	$2.51\times10^5 - 1.45\times10^6$
LC_{90}	7.86×10^7
95% limits	$3.04\times10^7 - 8.11\times10^8$
slope ± SD	0.705 ± 0.100
χ^2	0.128
df	3
H	0.04

Efficacy of *B. bassiana* in field treatments

The evaluation of the field treatments showed no statistical differences between the mortality of *P. sylvestris* trees in all experimental variants ($H = 5.473$; $p = 0.0748$) (Tab. 2). On the plots treated with *B. bassiana,* a colonisation of 13% and 11% of trees sprayed with isolates, respectively, WG and BB1, as well as 14% of untreated trees, was found.

Only living larvae of *P. castaneus* were found in the stems of observed trees. There were no statistically significant differences between the numbers of larvae developing in the treated and untreated fungus ($H = 0.1305$; $p = 0.9368$) (Tab. 2). In all the variants of treatment, there were from 7 to 16 larvae in the lower parts of stems with a length of 20 cm.

Table 2. Colonization of trees treated with *Beauveria bassiana* by *Pissodes castaneus*

Fungus strain	Mean (± st. dev.) numbers of dead trees	Mean (± st. dev.) numbers of larvae found on the stem section (20 cm length)
WG	10.8 ± 2.1	9.9 ± 2.5
BB1	8.6 ± 1.9	10.6 ± 1.6
control-untreated	11.6 ± 1.8	12.1 ± 2.3

Discussion

Research on the use of natural enemies to limit the number of *Pissodes* weevils have been concentrating mainly on the biology of parasitoids and also included a discussion on the possibility of their use in the form of introduction to the areas of pest increased occurrence (Kenis and Mills 1994; Kenis et al. 2004). So far, the papers published by Alauzet (1987, 1990) and Kenis et al. (1994, 1996, 2004) contain most information on the parasitoids of *P. castaneus*. Whilst little information concerns the pathogens of *Pissodes* weevils, mainly white pine weevil *Pissodes strobi* (Peck), which, especially in North America, is a dangerous pest of white pine (*Pinus strobus* L.) and Norway spruce [*Picea abies* (L.) H. Karst] (Alfaro *et al.* 1995). Streett *et al.* (1975) described the morphology of microsporidia *Nosema* sp. isolated from death and alive larvae of *P. strobi* from the population occurring in Maryland, USA. At the same time, they suggested the use of these microorganisms to reduce the number of pest. There is also the lack of studies to use the entomopathogenic fungi against *Pissodes* weevils. Only Trudel *et al.* (2007) made laboratory attempts to assess the potential use of *B. bassiana* to limit the population of *P. strobi*. Thus, the presented results of experiments on the use of *B. bassiana* in the forms of prepared in the laboratory conidia suspensions and commercially available bioproduct 'BoVeril' to reduce *P. castaneus* population are the first information of this type.

The laboratory results showed a mortality of over 90% of *P. castaneus* beetles feeding on twigs treated with different concentrations of *B. bassiana* conidia. Similar results were obtained by Trudel et al. (2007), who observed nearly 90% mortality of *P. strobi* beetles feeding on the sections of *P. strobus* twigs treated with Canadian isolates of *B. bassiana*. Comparison of LC_{50}

obtained in presented results and those found in Trudel's *et al.*(2007) studies pointed at the higher activity of Polish isolate. The higher activity of *B. bassiana* in laboratory conditions was confirmed in the studies pointed at its use in limiting the number of the large pine weevil *Hylobius abietis* L. – another curculionids feeding on young coniferous trees. Wegensteiner and Führer (1988), who analysed the mortality of *H. abietis* beetles infected by the fungus in laboratory conditions found 100% mortality of insects feeding on a bark of Norway spruce treated with 1.17×10^{8}conidia cm^{-2} bark. Similar tests were made by Ansari and Butt (2012), who observed 100% mortality of *H. abietis* beetles during 3 weeks after infection with *B. bassiana* at the concentration 1×10^{8} conidia ml^{-1}.

Despite the high activity of *B. bassiana* in the laboratory, the results of field trials showed no effect of fungus treatment on the infestation of Scots pines by *P. castaneus*. The lack of similar field experiments described in the literature makes the discussion of obtained results difficult. Most of the field trials limiting the number of pest forest insects with *B. bassiana* was related to species such as *H. abietis* and spruce bark beetle *Ips typographus* L. The results of these tests confirmed the low efficiency of pathogen in the protection of forest against pest insects. Wegensteiner and Führer (1988) paid attention to very low mortality of *H. abietis* beetles attracted to the spruce bark treated with *B. bassiana*. Williams *et al*. (2013) tried to reduce the numbers of *H. abietis* larvae developing in the stumps through the application of *B. bassiana* together with entomopathogenic nematodes (*Sterneinema carpocapsae* and *Heterorhabditis downes*) on these stumps. The results showed a higher effectiveness of nematodes (up to 50% mortality of larvae) compared to very low efficacy of fungus (up to 3.5% mortality of larvae).

Experiments on the use of *B. bassiana* in relation to the spruce bark beetle were conducted in Poland since the 1960 of last century, but the field application of the pathogen did not give satisfactory results (Bałazy 1962; Cichońska and Świeżyńska 1993; Głowacka and Świeżyńska 1993; Malinowski 2011). The studies carried out by Grodzki and Kosibowicz (2015) confirmed the low efficacy of *B. bassiana* applied in powder form in pheromone traps to capture and thereafter release fungus-infected bark beetles to the forest environment. Also direct spraying or dusting of lying trap logs made from *P. abies* did not have any effect on spruce infestation by *I. typographus,* its reproduction success and development or natural enemies inside the bark. Whilst in Slovakia, spraying of standing trees infested by *I. typographus* leaded to 28% mortality of pest caused by pathogen (Jakuš and Blaženec 2011).

Thus, the literature data and the results of field experiments presented in this publication do not recommend the use of *B. bassiana* in forest protection against small banded pine weevil. However, promising results of laboratory experiments may provide a basis for further research to achieve greater effectiveness in the field treatments.

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