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An attempt to use the fungus *Beauveria bassiana* (Bals.) Vuill. in forest protection against the bark beetle *Ips typographus* (L.) in the field

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Abstract. In 2011–2013, trials on the use of the entomopathogenic fungus *Beauveria bassiana* against bark beetle (*Ips typographus*) populations were carried out under open field conditions in Norway spruce stands suffering from an outbreak in the Beskid Żywiecki Mts. in Poland. Modified pheromone traps were deployed to capture and thereafter release fungus-infected bark beetles to the forest environment. Infested spruce trees felled next to the traps remained unaffected by the transmission of the fungus to insect populations. Direct spraying or dusting of lying trap logs and suspended caged rearing bolts did not have any effect on spruce infestation by *I. typographus*, its reproduction success and development or natural enemies inside the bark.

A very small effect on mortality rates of target as well as non-target insects overwintering in the dusted litter was observed. Treated stands, unlike control stands, were indirectly affected by the treatment, evidenced by the reduction of tree mortality due to bark beetle infestation.

At present, no recommendations concerning the potential use of the fungus in forest protection can be given. However such an environmentally friendly approach represents a promising future prospect.

Keywords: bark beetles, *Picea abies*, biological control, montane forests, field trials

1. Introduction

Mountain forests in Poland are characteristic of a high, and often dominant share of Norway spruce, which results in permanent or else periodically escalating threat posed by cambiophagous insects, and, especially the European bark beetle *Ips typographus* (L.). Currently applied control measures to decrease exceeding populations of this pest are labour-consuming and costly, thus there always is a need for seeking new methods assuring higher pesticide efficacy with less effort. A promising direction to take can be the use of entomopathogenic organisms, and, especially the fungus *Beauveria bassiana* (Bals.) Vuill., which is a natural control agent that fits the now implemented model of forestry based on ecological principles.

Already in the 1930's, Karpiński (1935) reported *B. bassiana* as the reason of *I. typographus* mortality. Bałazy (1962) isolated the fungus from dead imagines of several beetle species, including bark beetles feeding on Norway spruce and comprehensively described the relationships between bark beetles and pathogens, including *B. bassiana* (Bałazy 1966, 1968; Bałazy et al. 1967). When concluding on 20-year-long studies, Głowacka and Świeżyńska (1993) listed only two entomopathogenic fungal species associated with *I. typographus*. The fungus *B. bassiana* was highlighted as one having a wide range of forest insects as host organisms. On the other hand, however, low efficacy of entomopathogenic fungi under natural conditions has been pointed out, for example, Bałazy (2012) reported only 0.7–3%. reduction in the population of *I. typographus* young generation. Laboratory studies on *B. bassiana* infection of bark beetle adults and larval stages indicated adequate effectiveness of this fungus biopreparation and high bark beetle mortality in closed cultures (Bałazy 2013). The results of the study on efficacy of the pathogen and its capability to spread out, carried out under laboratory conditions in the years 1990–2004 (Wegensteiner 1992, 1996; Markova, Samshinyakova 1990; Markova 2000; Kreutz et al. 2004a,b), allowed to elaborate *B. bassiana* application technique, later tested under field conditions (Vaupel, Zimmermann 1996; Kreutz et al. 2000, 2001, 2004b). In Poland, the trials on

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B. bassiana spray treatments on Norway spruce logs brought unsatisfactory results (Cichońska, Świeżyńska 1993). On the other hand, positive results were obtained in the trials conducted in the National Park Šumava in Czech Republic (Landa et al. 2007, 2008), as well in Slovakia (Kunca et al. 2009, Vakula et al. 2010). In 2007, in Slovakia, there were also carried out tests on *B. bassiana* water suspension applied on cut down bark beetle infested trees. The results obtained showed almost 29% infection in the pest population studied (Jakuš, Blaženec 2011).

Several biopreparations have been tested against *I. typographus* when combined with the use of pheromone traps (Vaupel, Zimmermann 1996). Good results were obtained for Boverol (containing *B. bassiana* spores) when tested under semi-natural conditions (cages with bark beetle rearing situated in forest environment) (Kreutz et al. 2000, 2004b). However, under natural conditions, Boverol treatments combined with the use of modified pheromone traps showed no expected direct effects (Vakula et al. 2010). Nonetheless, in the years 2009–2011, Boverol treatments performed along with the use of other pest control measures applied in active forest protection helped to stop the progress of *I. typographus* outbreak in military forests located in central Slovakia (Vakula et al. 2012). Other biopreparation - BoVeril - was preliminarily tested with no successful results in Poland (2009) in the Beskid Śląski, Beskid Żywiecki and Beskid Sądecki mountains (Grodzki, not published data). The same biopreparation was also evaluated in Slovakia (2008) and no positive results were obtained as well (Kunca et al. 2009).

In view of the aforesaid inconclusive results, there was recognised a need to further evaluate the possibilities of using biopreparations based on *B. bassiana* as a control agent under the conditions of managed forests affected by *I. typographus* outbreak. Employment of the fungus as a means of biological control could be of most importance, especially in mountain forest stands that often are subject to many restrictions with regard to pest control methods because of environmental concerns (Sierpińska, Grodzki 2012).

Taking into account that up to date, the trials have been generally conducted under laboratory conditions, the main goal of the present study is to determine whether at all and to what extent there functioned the entomopathogenic attribute of *B. bassiana* when artificially introduced into managed forests. It was assumed that the results could give a basis for evaluation of prospects for utilisation of this fungus in the active protection of forests. The study comprised testing different methods of biopreparation application, evaluating treatment efficacy (directly - through the assessment of *I. typographus* mortality, and indirectly - through the assessment of tree mortality) and appraising biopreparation selectivity (the effect on non-target insects).

2. Study area and methods

The study area was situated in the Beskid Żywiecki mountains, where active *I. typographus* outbreak started in 2002 and culminated in 2007–2008 (Grodzki 2010). In view of specifics of this study, the trials were set up outside Natura 2000 sites established in the region (PLH240006 Beskid Żywiecki, PLH120001 Babia Góra). The forest complex selected for *B. bassiana* treatments was situated within the area of the Forest District Jeleśnia, the forest division Koszarawa Bystra, management units: 43 and 44 (49º39'12"N, 19º26'15"E). The area is situated on southern slopes of Lachów Groń peak (1045 m above sea level [a.s.l.]), below the alp Hala Janoszkowa, at altitude approximately 1000 m a.s.l. The forest complex comprises mountain forest and mixed forest (fresh and wet) sites. Most often, these are 90–110 years old stands of low stem density and 70–100% share of Norway spruce, and beech stands with spruce and fir admixture. All through the observation period, in the stands tested, there were applied standard control measures used in the protection of forests against *I. typographus*, such as setting up pheromone traps as well as identification and felling (debarking) of infested trees before the emergence of young generation.

Field trials on biopreparations containing *B. bassiana* spores were carried out based on the permission granted by the Minister of Agriculture and Rural Development (Decision R-657/2011b). Fungus treatment timings were synchronised with the time of the occurrence of *I. typographus* developmental stages, that is, adults when swarming and channelling out galleries at some point in the vegetation season and afterwards - the stages overwintering in the litter. For the treatments carried out in the years 2011–2012, there was used BoVeril - water soluble granulate preparation (Bioved 2005 Kft., Hungary), containing spores of *B. bassiana* strain Bb1 at the concentration 5×10^8/1g (vitality min. 65%), promoted by the producer as appropriate for control of the European bark beetle. According to the label, preparation efficacy depends on storage temperature (1 year at 5–10ºC or 2 years at 18ºC) as well as on air humidity and temperature during the treatment (needed to maintain fungal entomopathogenic activity: 60–70% and 12–26ºC, respectively). In 2013, on a limited scale, there was tested biopreparation Boverol (Fytovita, Czech Republic), obtained from the company Fytofarm Ltd. (Slovakia), containing *B. bassiana* spores (strain Bba 138) at the concentration 1×10^7/g. The label of the latter did not provide information concerning specific application requirements.

In the years 2011–2013, in the stands studied, there were installed 15 pheromone traps (Photo 1), which were modified to allow attracted *I. typographus* specimens getting out to continue their flight after contact with the fungal biopreparation tested. Pheroprax dispensers (BASF) were used as baits and were exchanged just about every 6 weeks.

The effects of *B. bassiana* biopreparation treatments on *I. typographus* population were evaluated every year, through the analysis of an infestation level both in standing Norway spruce dying trees and trap logs. In the years 2011–2012, dying tree infestation was evaluated on three parts of the tree trunk: I - the stem base, II - the middle part between the butt

Photo 1. Pheromone traps containing dry BoVeril for the infection of *I. typographus* beetles: MK-type trap (a), element with fungal preparation (b), Ekotrap-type trap (c)

and the crown and III - under the crown (Grodzki 2007). After tree felling, the tree trunk was divided into sections, their perimeters were measured and the galleries of *I. typographus* and other bark beetles were counted. The assessments were carried out every year, both in the tree stands treated with *B. bassiana* and in the control stands located 13–15 km away (in 2011: forestry management unit 223b in the forest division Romanka Dolna; in 2012: 108f, forest division Korbielów; 2013: 196ab/199, forest division Romanka Dolna). In 2012, a comprehensive assessment of sex ratio was carried out in *I. typographus* population infesting the trees studied (the number of mating chambers was assumed as the number of males and the number of maternal galleries as that of females). There were also assessed: the length of 10 galleries/tree trunk section; bark beetle fecundity (the number of eggs and developing larvae/gallery channelled out by 1 female in the same 10 galleries analysed) and *I. typographus* mortality in galleries due to parasitoids and entomopathogenic fungi.

In the middle of May 2013, both within tested and control stands, there were displayed trap logs (5 m long, pruned Norway spruce logs with no pheromone dispenser attached). In the middle of July 2013, bark beetle infestation levels were analysed on the traps. The assessments were carried out on two log sections (0.5 m far off from log ends) which were debarked on the surface area of 0.5 m × 1/2 of perimeter length. The number of *I. typographus* galleries was assessed on 1 dm^2 of the bark section.

On 7 July 2011, there were performed direct spray treatments with the use of BoVeril. Within the area of the management unit no. 44 (forest division Koszarawa Bystra), there were felled three Norway spruce trees, then pruned and sprayed at the rate of 500 g BoVeril/24 l water with the use of a brand new backpack sprayer (Solo 425). Then the logs were covered with fresh spruce branches. Pheromone dispensers (Pheroprax) were attached to each log so as to assure appropriate trap infestation by *I. typographus* beetles. The treatment was performed at air temperature of 19ºC and relative air humidity of 65% (data from the weather station Koszarawa Tajch). At the same time, next three tree traps were prepared and displayed outside of the stand, for later treatment after presumed infestation by *I. typographus*. On 26 July 2011, the traps covered with branches were sprayed once again (without removing the branches). This treatment was performed at air temperature of 18ºC and relative humidity of 68%, partial cloud cover and light rain sprinkles. There was used 12 l of water solution with 200 g of BoVeril. Bark samples from the logs sprayed before and after infestation were taken on 29 July 2011. The samples were preliminarily evaluated under field conditions, and then placed in the so-called wet chambers (Petri dishes with wetted filter paper) to allow further development of *I. typographus* beetles under laboratory conditions. At the same time, at air temperature of 16.3°C and relative air humidity of 110%, there were sprayed (200 g BoVeril/12 l water) three tree traps (with no pheromone dispenser) earlier displayed and infested. The traps were covered with spruce branches and left outside of the stand until the emergence of next adult generation (fungus dispersion in *I. typographus* population was anticipated).

On 16 May 2013, there were performed Boverol sprays on two displayed tree traps. The treatment was carried out at air temperature 18.3°C and relative air humidity 53%, with the use of Solo 425 sprayer and biopreparation water solution (500 g/5 l of water). Other two tree traps were dusted with Boverol dry formulation. Pheroprax dispensers were attached to both, sprayed and dusted traps, and then the traps were covered with fresh spruce branches and left until 8 August 2013, when the level of trap colonisation by insects and their developmental stages were assessed.

In the years 2012 and 2013, the infection of *I. typographus* beetles by *B. bassiana* spores was evaluated under semi-natural conditions. In order to do so, nine 0.5 m-long fresh Norway spruce bolts were cut out and suspended in three groups (three bolts/group) on a rope mounted between standing trees. The bolts in each group were covered with muslin sacks (Photo 2a). Into each sack, there were released 50 alive adult *I. typographus* beetles, which were earlier captured (22 May 2012 and 14 June 2013, respectively) within other forest district area, using Pheroprax and muslin screen. In each of the three groups, *I. typographus* adults were released in line with the following pattern: one of three bolts in the group was infested with adults covered with Boverol dry formulation and two remaining bolts - with not contaminated adults. After 3–4 weeks, (on 21 June 2012 and 5 July 2013), biopreparation water solution was injected with a syringe (Photo 2b) into *I. typographus* entrance holes found on the bolts. In each group, syringe treatments were performed on the second of three bolts. The third bolt in each group remained untreated as the control. The bolts stayed suspended until the end of the vegetation season, and then *I. typographus* gallery density and gallery development degree as well a level of bark beetle infection by *B. bassiana* were assessed.

In the fall of 2012, in the stand selected for the treatments, there was established a trial with the aim to determine the effect of *B. bassiana* on *I. typographus* beetles overwintering in the litter. There were selected five Norway spruce stumps (remains of bark beetle infested Norway spruce trees, cut down in the late fall), which were surrounded by bark pieces falling down from the trees due to woodpecker feeding. It was assumed that a number of *I. typographus* adults will leave the bark in order to overwinter in the litter (Onyśko, Starzyk 2011). Emergence traps (ground photo-eclectors) were displayed in pairs (Photo 3): one on the litter dusted with BoVeril preparation and the second one on the untreated litter (control). Altogether, five pairs of the traps were displayed. The trial was repeated in 2013 (early spring), before *I. typographus* swarming, but this time, the litter was treated with Boverol preparation (dry formulation). The analysis of insects found in the traps was carried out in July 2013.

Weather conditions during the observations carried out in the year 2011 were monitored based on data collected by the meteorological station Koszarawa-Tajch (County Office Żywiec), situated in the neighbourhood of the study area (downloaded from www.traxelektronik.pl). In the years 2012–2013, air temperature and humidity were monitored on site by the authors of the study with the use of TinyTag Extra (in 2012) and Davis Vantage VUE (in 2013) measurement devices.

a

b

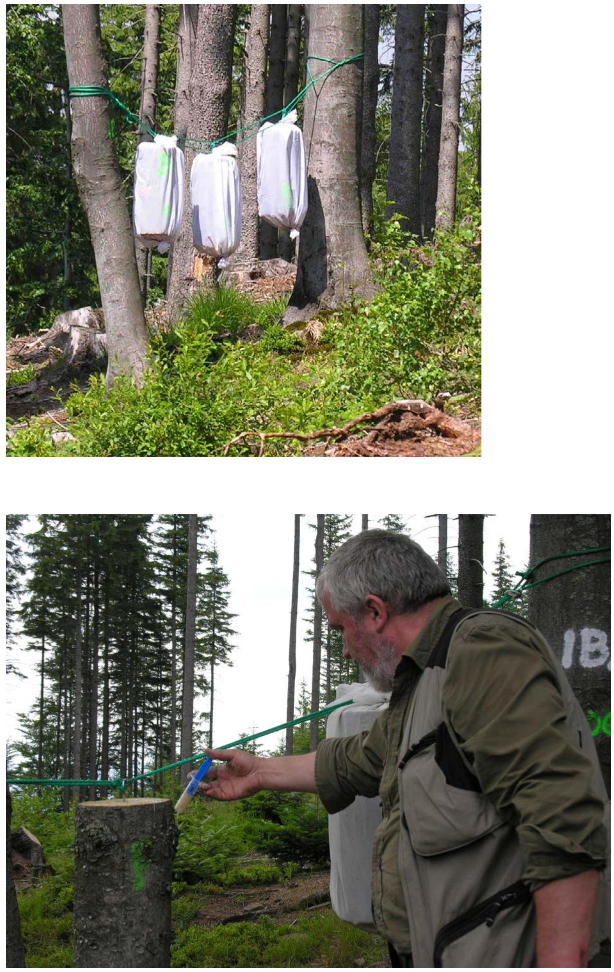

Photo 2. Suspended spruce bolts for the experiments with direct infection of beetles (a) and the injections of fungal dilution into the *I. typographus* gallery systems (b)

Photo 3. Ground eclectors for overwintering beetles exposed on the litter in the spring 2013

The evaluation of indirect biopreparation effects was based on the analysis of the intensity of spruce mortality due to *I. typographus* infestation both within the study and control Norway spruce stands. Two forest complexes were selected for the analyses: one with an area of 80.65 ha in the forest division Koszarawa Bystra (management units: 41–44) and one with an area of 92.49 ha in the forest division Sopotnia Potok (management units: 187, 189–191). The stands observed were situated in comparable growth environments (southern exposure, approximately 1000 m a.s.l.). The analysis of data concerned the years 2009–2013, and this period comprised both the years before the trials on *I. typographus* control treatments with the use of *B. bassiana* and the years when the observations were carried out (2009–2010 and 2011–2013, respectively). Data on the volume of felled trees infested by *I. typographus* were obtained from the Forest District Jeleśnia (state forests database – SILP).

Differences between the mean values obtained for the density of bark beetle galleries, length of maternal galleries and offspring numbers in galleries assessed in the treated and control stands were statistically tested using non-parametric Mann-Whitney U test. All calculations were performed using MS Excel and Statistica v. 5.0 (Stat-Soft, Inc. 1997).

3. Results

Weather conditions during observations carried out in the vegetation season

In 2011, in the period when *B. bassiana* treatments against *I. typographus* were carried out (subsequent sprays during tree infestation when fungal infection of insects was expected), local conditions were characteristic of cold and rainy weather, with low (10–12ºC) night temperatures and relatively high precipitation (Table 1).

In the following years (2012–2013), weather conditions were comparable (Table 1). At the beginning of May 2012, air temperature fluctuated, and there were occasionally observed quite cool days (slightly above 5°C). In the middle of the month, temperature dropped below 5°C. Short-term warming occurred in the third decade of May, which allowed collecting *I. typographus* adults merely at their late swarming. Later in the season, the weather changed but higher temperatures (up to 31°C) and lack of precipitation were observed only after the middle of June. At the time when *I. typographus* galleries were established on Norway spruce bolts and during biopreparation syringe injections into adult entrance holes, the weather conditions were fine, with moderate temperature and lack of precipitation.

In the spring of 2013, meteorological conditions changed likewise, and in May and June, there were observed cold and rainy periods (Table 1). This affected the timing and duration of *I. typographus* swarming, which, in turn, caused difficulties in adult collection followed by a delay in establishing the trials planned. The weather changed later in the season, however, with no negative effects on the development of pest offspring in galleries channelled out during good weather conditions (temperatures up to 27°C). At the time of biopreparation syringe injections into experimental Norway spruce bolts, weather conditions were advantageous as well.

Pheromone traps with fungus biopreparation

I. typographus adults were contaminated with *B. bassiana* spores when they were leaving the pheromone traps with fungal biopreparation added. At the same time, too much biopreparation on bark beetle body resulted in death of insects due to the clogging of trachea. In the second and third year of observations, insect mortality due to the latter factor was partially halted through adding a foam element to the traps with biopreparation (Photo 1b). The adults leaving the traps flew away and only a small number of the insects observed fell down and resided in the litter around the traps.

At the end of August 2011, in the stand treated, there were analysed 27 bark sections taken from 9 Norway spruce trees dying due to *I. typographus* infestation. In the control stand, there were analysed 21 bark sections from 7 Norway spruce trees. The results showed that in the stands treated, mean infestation density in dying trees was 1.541 (± 0.520 SD)/feeding site on 1 dm^2, whereas in the control, mean infestation density was 0.497 (±0.289 SD)/dm^2 (Fig. 1). The differences were statistically significant (M–W U = 16.0; z = 5.56; $p < 0.001$).

In the stand treated, in Norway spruce bark sections of the size 25 × 25 cm, there were found from 0 to 90 alive *I. typographus* beetles, (on average 15.37 specimens) and from 0 to 90 pupal chambers (on average 16.11). In eight bark sections (30% of the total number of sections analysed), there were also observed dead bark beetle adults - approximately four specimens/section. Among dead *I. typographus* beetles,

Table 1. Air temperature and relative humidity in the area and period of experiments in 2011–2013

Data concerning period:	Air temperature (°C)			Air relative humidity (%)			Date of spraying / dusting
	mean	min.	max.	mean	min.	max.	
5.06.–22.06.2011	17.3	10.1	31.7	76.8	28.7	100.0	7 and 21.06.
1.05.–20.06.2012	12.4	-0.7	31.0	74.7	20.0	100.0	22.05.
1.05.–20.06.2013	12.5	1.9	26.9	81.5	45.0	100.0	16.05. and 14.06.

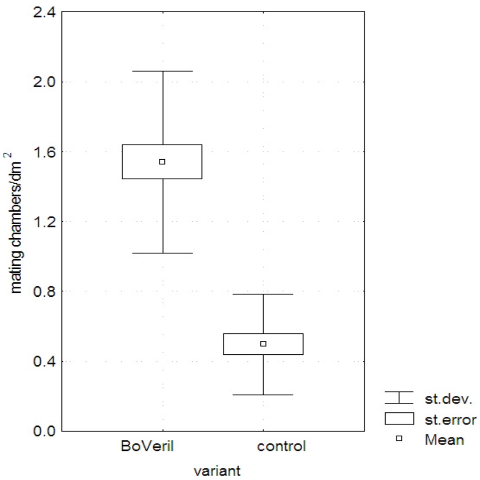

Figure 1. Mean infestation density of spruce stem sections in the subcomp. 43ab/44b (experiment) and 223b (control) in Jeleśnia Forest District in 2011

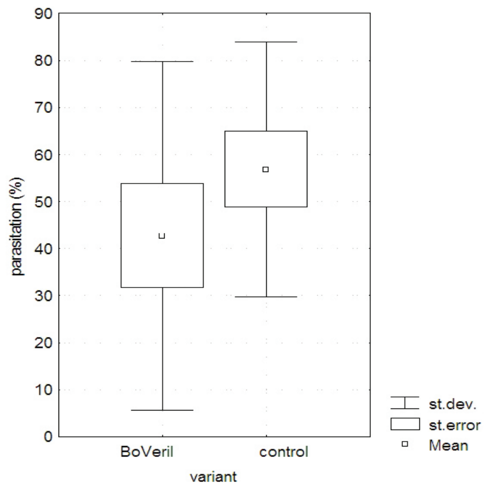

Figure 2. Mean parasitation (%) of *I. typographus* by *Coeloides bostrychorum* in the stem sections of spruces in the subcomp. 43ab/44b (experiment) and 223b (control) in Jeleśnia Forest District in 2011

Photo 4. *Coeloides bostrychorum* cocoons in *I. typographus* galleries found on infested standing trees in the stand subjected to the experiments

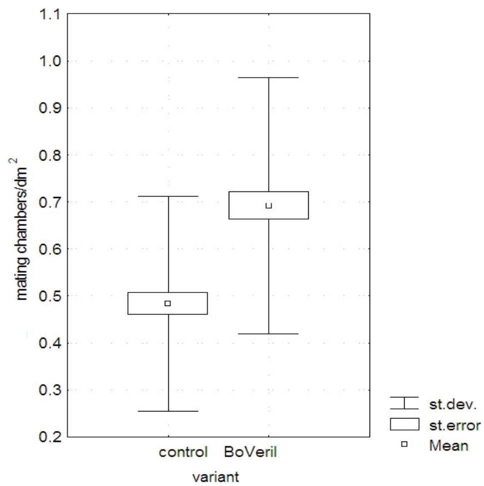

Figure 3. Infestation density by *I. typographus* in the stem sections of standing spruce trees in 2012: control – Korbielów 108f, BoVeril – Koszarawa Bystra 43a/44b

there was only one overgrown by fungal mycelium. In 11 (41%) out of 27 bark sections analysed, there were observed cocoons of the parasitoid *Coeloides bostrychorum* (Gir.) (Hym., Braconidae) (Photo 4). A level of *C. bostrychorum* parasitism in *I. typographus* ranged from 10 to 100% in the stand treated. It was, on average, lower than that observed in the control stand (10–90%) (Figure 2). Collected parasitoid pupae in cocoons continued their development and successfully emerged as imagines under laboratory conditions.

In 2012, there were carried out in depth examinations of four dead Norway spruce trees cut down in the vicinity of the pheromone traps with *B. bassiana* spores (management units 43a/44b) and of three control spruce trees (management unit 108f). The results showed higher average density of *I. typographus* galleries in the treated stand (0.692 ± SD 0.272) than that observed in the control (0.484 ± 0.229). The differences were statistically significant (M–W U = 1752.0; $z = -5.97$; $p < 0.001$) (Fig. 3). Maximal gallery density exceeded 12 mating chambers/dm^2.

The analysis of selected parameters of *I. typographus* galleries showed no statistically significant differences between the length of maternal galleries observed in the stand treated and that in the control stand (7.469 and 7.184 cm, respectively) (Fig. 4a). At the same time, the number of offspring per one female in the control stand was slightly (not significant difference) higher than that in the treated stand: 22.75 and 20.92, respectively (Fig. 4b).

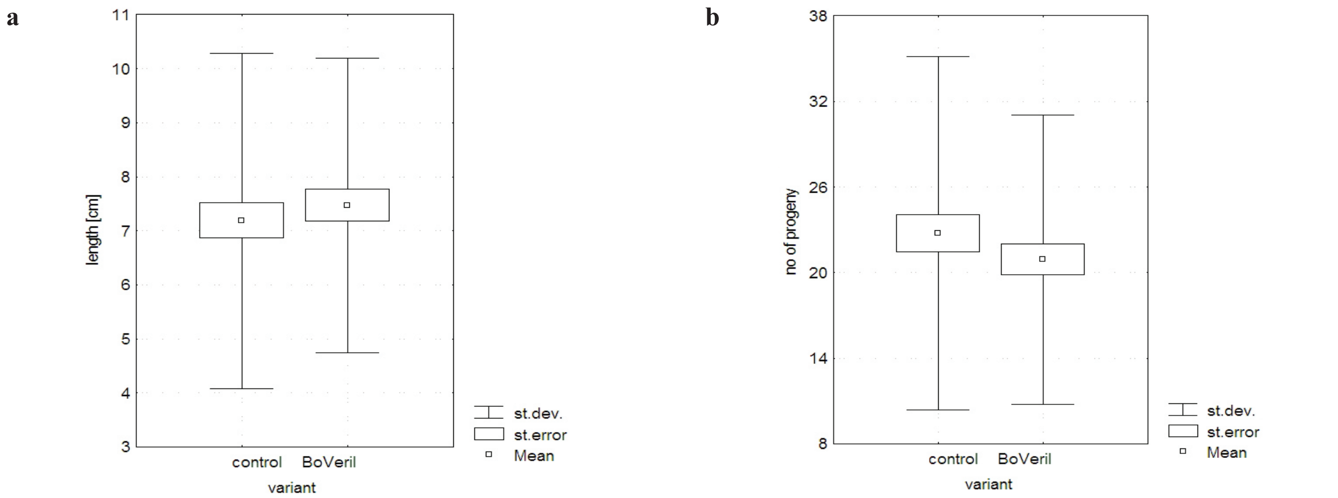

Figure 4. Mean maternal gallery length (a) and number of progeny from individual female (b) in the stands treated by BoVeril (Koszarawa Bystra) and control ones (Korbielów) in 2012

The sex ratio in the population of *I. typographus* infesting dying trees was comparable in the treated and control stands. In both cases, the percentage share of females was to some extent higher than that of males - on average: 61% (ranging from 50–79%) in the treated stand and 62% (56–65%). The galleries with one or two maternal galleries dominated in the treated and control stands (92 and 96%, respectively), whereas the share of galleries with four maternal galleries was maximum 1%.

Apparently, *I. typographus* successfully accomplished all developmental stages since in the galleries observed, there were found pupae and immature yellow adults. No traces of *B. bassiana* infection were found. On the other hand, there was observed high *C. bostrychorum* parasitism level (80%) in the analysed *I. typographus* population. In case of parasitoids, no signs of fungal infections were observed whatsoever.

In August 2013, perfunctory analyses carried out in the treated stands concerned *I. typographus* infestation levels in standing Norway spruce trees. The outcome of surveys confirmed the results obtained in 2011–2012. On relatively small numbers of dying trees, there were observed typical patterns of infestation by *I. typographus*, *I. amitinus* (Eichh.) and *Pityogenes chalcographus* (L.), with the full spectrum of bark beetle development stages (including alive imagines). The presence of parasitoids was recorded along with that of *Thanasimus formicarius* (L.) predatory larvae and Staphylinidae beetles. Then again, no traces of insect fungal infections were found in the feeding sites analysed.

In the summer of 2013, there were also carried out the assessments of *I. typographus* infestation in 40 sections of bark beetle lying trap logs, displayed both in the treated and control stands (10 traps in each). In the treated stand, 10 (50%) out of 20 sections of trap logs were infested by *I. typographus,* and in the control stand - 11 sections (55%). Average density of trap infestation was higher in the control stand (0.373 ± SD 0.588 galleries/dm^2) compared to the Boverol-treated stand (0.207 ± 0.325 galleries/dm^2); however, the difference was not statistically significant (Fig. 5). The trap logs were also colonised by *P. chalcographus*, whose feeding signs were observed in 13 (65%) out of 20 analysed trap sections in the treated stand and in 6 (30%) out of 20 trap sections analysed in the control stand. Average density of *P. chalcographus* gallery systems/trap dm^2 was higher in the control stand compared to the treated stand (0.504 ± SD 0.822 and 0.407 ± SD 0.534); however, the difference was not statistically significant. In no way, *P. chalcographus* specimens with signs of fungal infection were observed.

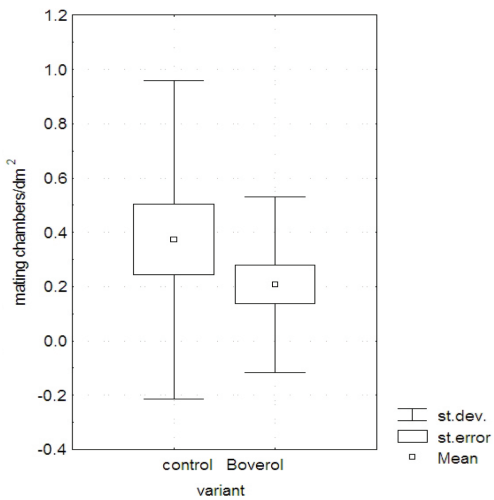

Figure 5. Infestation density by *I. typographus* on lying trap logs in 2013: control - Romanka Dolna 196ab/199a, Boverol - Koszarawa Bystra 43a/f

Evaluation of the effect of direct contamination of *I. typographus* adults with *B. bassiana* spores

In the galleries channelled out in the bark samples collected on 29 July 2011 from the trap logs treated with Boverol biopreparation, there were found healthy *I. typographus* larvae and just only one adult specimen with visible signs of fungal infection (overgrown by white mycelium). Trap logs that were treated with Boverol water solution in May 2013 and examined on 8 August 2013, showed high level of *I. typographus* infestation as well extensive colonisation by *P. chalcographus* (except for upper log parts with dried out phloem tissue). At the same time, there were observed feeding signs of longhorn beetles - *Rhagium* sp. and *Monochamus* sp. No signs of insect fungal infections were observed in bark beetle galleries. Bark beetles feeding on the trap logs must have gone through all developmental stages, since there were observed adult exit holes. In case of *I. typographus*, there were found characteristic signs of adults' maturation feeding, the so-called 'deer horns'. Generally, in *I. typographus* galleries, there were observed alive *T. formicarius* larvae. Similar patterns of insect infestation and colonisation were observed on the trap logs treated with Boverol dust formulation; however, there were found more than a few adult *I. typographus* infected with fungal mycelium (Photo 5), but these constituted just a minute fraction of 1% of the overall population that developed on the tree traps analysed.

In 2012, *I. typographus* infestation was observed in all variants of the trial on suspended Norway spruce bolts. Maternal gallery density ranged from 0.28–0.64/1 dm^2. The highest mean density was observed in the control treatment (0.52) compared to that in the spruce bolts treated with Boverol solution injected into bark beetle entrance holes (0.48) and dust formulation treatment (0.41); however, this pattern was not observed in every repetition of the experiment (Fig. 6a). Same variability was observed for the numbers of bark beetle exit holes found on the bolts tested (means obtained: 5.46 - control, 4.82 - injection, 4.36 - dust formulation, Fig. 6b). It was found that *I. typographus* went through all developmental stages, completed maturation feeding and produced

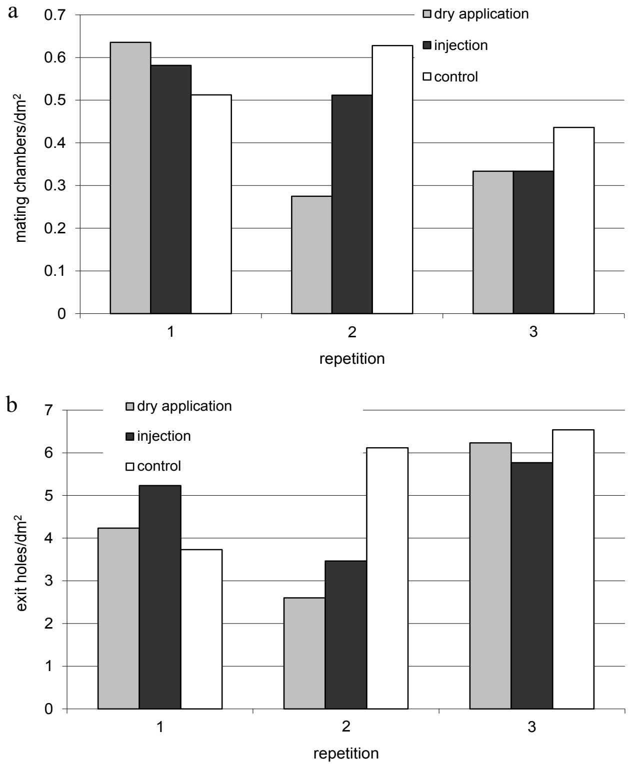

Figure 6. Number of mating chambers (a) and exit holes (b) on 1 dm^2 of bark area in individual experimental variants and repetitions in 2012

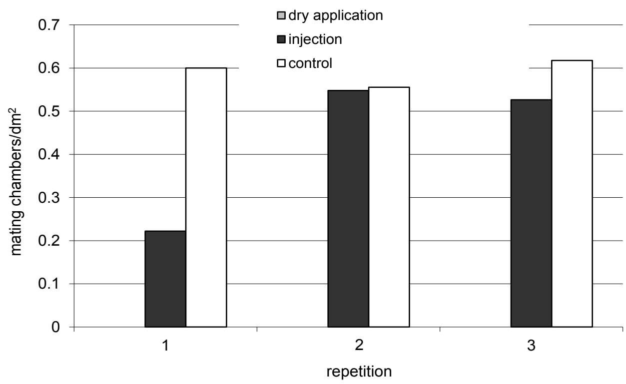

Figure 7. Number of mating chambers on 1 dm^2 of bark area in individual experimental variants and repetitions in 2013

next generation (in November, there were observed numerous alive adults, the part of which channelled out shallow chambers for overwintering in the bark). No differences in bark beetle development rates were found between the treatments tested.

In 2013, the mean number of maternal galleries on control Norway spruce bolts (0.59) was higher than that in Boverol injection treatment (0.43) (Fig. 7). Then again, this pattern was not observed in all trial repetitions. At the same time, in the spruce

Photo 5. *I. typographus* adult penetrated by mycelium

bolts treated with Boverol dust, in two out of three, there were found four entrance holes of males who started to engrave mating chambers (situated close to bolt tips) but died, and no signs of bark beetle infestation was noted on the third bolt. The analysis of larval galleries (established around five randomly chosen maternal galleries/Norway spruce bolt/treatment) showed no larvae development in Boverol dust treatment, and 12–46 or 24–44 larval galleries in Boverol injection and control treatments, respectively.

The trial on overwintering insects in 2011/2012 resulted in collection of altogether 74 insects from five pairs of ground photo-eclectors (20 specimens in the treatment and 54 in the control) (Table 2).

In the spring of 2013, no insects were found in ground photo-eclectors.

Accomplishment of forest protection aims - evaluation of Norway spruce mortality due to bark beetle infestation

Dynamics of Norway spruce mortality was analysed based on data on the volume infested by cambiophagous insects trees, harvested in selected forest complexes. According to the forest inventory data, in Koszarawa Bystra forest complex, the stands with 50% Norway spruce share were situated on an area of 42.97 (53% of the total complex area) while in Sopotnia Potok complex - on 82.44 ha (89% of the total area). Irrespective of the differences between the stands in these complexes, the intensity of spruce mortality due to insect infestation was quite similar. In the years 2009–2011, within the area of the two forest complexes jointly, there were annually harvested approximately 15.000 m^3 of infested spruce trees (in 2009, more logging was performed in the forest division Sopotnia Potok, and in 2010, in the forest division Koszarawa Bystra). In subsequent years, logging of dying trees gradually decreased, especially in the forest division Koszarawa Bystra (Fig. 8a). For the purpose of comparative analyses done in the present study, data on the volume of logged infested trees within the areas of similar sizes of selected management units were standardised,

Table 2. Number of insects found in ground eclectors exposed on litter for 2011/2012 winter

Taxon	Experimental variant	Control variant
Ips typographus (L.)	3	8
Orthotomicus laricis (Fabr.)	1	1
Dryocoetes autographus (Ratz.)	1	6
Pityophthorus pityographus (Ratz.)	-	2
Hylastes spp.	-	8
Otiorrhynchus sp.	2	1
Curculionidae (others)	5	-
Tetropium castaneum (L.)	3	-
Oxymirus cursor (L.)	-	1
Glischrochilus quadripunctatus (L.)	-	1
Rhizophagus spp.	1	19
Notiophilus biguttatus (Fabr.)	-	1
Elateridae	4	6
Total	20	54

and the values per 1 ha were used in the analysis (Fig. 8b). In the years 2009–2010, the intensity of spruce mortality was high in both forest complexes analysed, and ranged from 10 m^3 to more than 20 m^3 per ha . In 2009, in the stand situated in the forest division Sopotnia Potok (later selected as the control for the trials with *B. bassiana* treatments against *I. typographus*), the number of dying trees was almost twice higher than that in the forest division Koszarawa Bystra (later treated stands). In 2010, the intensity of spruce mortality was higher in Koszarawa Bystra forest complex (Fig. 8b). In the following years (2011–2012), that is, during the period of observations carried out in the framework of the present study, the intensity of spruce mortality visibly decreased in Koszarawa Bystra and considerably increased in Sopotnia Potok. Available data allow assuming that the pattern continued also in the year 2013.

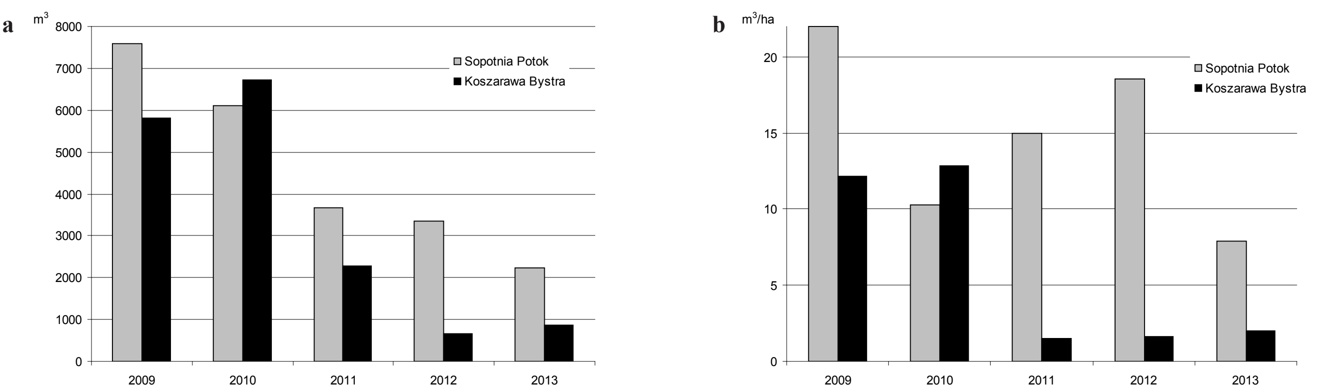

Figure 8. Volume of trees infested by bark beetles in Koszarawa Bystra (experiments in 2011–2013) and Sopotnia Potok (control) in 2009–2013: in total (a) and per 1 ha (b)

4. Discussion

The results of the field study on application of biopreparations containing spores of the entomopathogenic fungus *Beauveria bassiana* carried out during three subsequent vegetation seasons showed no transmission of the fungus into bark beetle population infesting Norway spruce trees. Also, no bark beetle mortality due to fungal infections was observed. The results obtained were quite surprising, since at least partial pest control was anticipated, based on up-to-date knowledge on entomopathogenic activity of *B. bassiana* against *I. typographus*, which has been confirmed by numerous laboratory as well as field trials. On the other hand, however, thus far, studies of *B. bassiana* and *I. typographus* relationships have not provided any sound basis for elaboration of pest control method useful in forestry practice.

Bałazy (1962) isolated *B. bassiana* from dead adult specimens of numerous insect species, including bark beetles occurring on Norway spruce. Among others, these were: *I. typographus*, *I. amitinus*, *P. chalcographus*, *Pityophthorus pityographus* (Ratz.), *Polygraphus poligraphus* (L.) as well as hymenopteran parasitoid *Rhopalophorus clavicornis* (Wesm.). The results of such studies indicated a possibility that artificial applications of biopreparation with *B. bassiana* spores should instigate fungal infections in numerous cambiophagous insects feeding on Norway spruce, as well as in parasitoid insects; however, this was not the case in the present study. Regardless of the treatment tested, both bark beetles observed as well as their parasitoids completed their development and produced new generations, whereas observed mortality due to the fungus tested was of no practical consequence.

Even though entomopathogenic activity of *B. bassiana* against *I. typographus* have been already confirmed many times under laboratory conditions, and bark beetle mortality observed in closed cultures was considerably high (Wegensteiner 1996; Kreutz et al. 2004a), fungal infections of *I. typographus* imagines, larvae and pupae (especially) occurred seldom under natural conditions. Also, insect mortality due to the infections only sometimes exceeded 3% of a given population (Bałazy 1966, 1968; Bałazy et al. 1967). Higher mortality was observed in young bark beetle generation at the time of maturation feeding or flying out, and especially in the populations overwintering in this developmental stage (Bałazy 2013). The results of the trials with the use of photo-eclectors situated in the litter (carried out at the turn of 2011 and 2012) confirmed the above to some degree. In the control treatment, there was found more than 2.5-fold higher number of bark beetles when compared to that observed in the treatment with fungal biopreparation onto the litter. This result can indicate biopreparation effectiveness against numerous beetle species (also predatory) overwintering in the litter, which could probably be due to better environmental conditions in the litter for the development of the fungus.

Bałazy (2013) calls attention to one case of very high (17%) proportion of *B. bassiana* infections in *I. typographus* population overwintering in dead trees. The results indicated higher effectiveness of the fungus against overwintering pest population, and this was somewhat confirmed by the result of the present study - in the trial on application of dust formulation of *B. bassiana* biopreparation. However, the results then obtained were difficult to interpret due to small numbers of insects available for collection in the first year of the study and total lack of insects in photo-eclectors observed next year. Then again, low insect numbers or lack of insects in photo-eclectors could be caused by trap location in the site with not many overwintering insects, which is quite possible in natural environment. Nonetheless, the results obtained showed no selectivity of biopreparation with regard to species from various systematic as well as functional insect groups.

Kreutz et al. (2001, 2004b) investigated the effects of artificially initiated fungal infections of *I. typographus* adults with the use of modified pheromone traps. The authors achieved transmission of the fungus into bark beetle galleries and observed a high degree of fungal infections. At the same time, a decreased intensity of bark beetle infestation as well as reduced length of galleries was observed. The trials were carried out under natural conditions, however, the cages with observed bark beetle prevented dispersal of the population tested, and therefore, the trial was actually carried out under somewhat controlled conditions. Under the conditions of the cages, there was possible to obtain almost 100% insect mortality (Kreutz et al. 2000), as well as fecundity reduction ranging from 23 to 29% (Vaupel, Zimmermann 1996). Under natural conditions of the present study, none of the aforesaid effects were observed, most probably due to the fact that dispersal of infected bark beetles did not allow to further investigate true effects of fungal biopreparation applied. On the other hand, there was observed considerably high (80%) level of parasitism in *I. typographus* population tested, which was mainly attacked by *Coeloides bostrychorum*. The latter showed no signs of fungal infections as well. The numbers of oviposited by the bark beetle eggs and that of its larvae were not lower than in the control stands, and eventual reduction might probably be connected with high gallery density in *I. typographus* infested trees situated in not treated stands (consequently, with some competition mechanism). What is more, no reduction of *I. typographus* fecundity was observed in the trial on muslin covered Norway spruce bolts, in which case infected and not infected *I. typographus* populations were separated. It is possible that the results obtained were due to relatively low resistance of biopreparation to environmental factors (high temperature, inadequate humidity), although during application, proceeding in line with the guidelines on biopreparation label was ensured.

According to Zimmermann (2007), under laboratory conditions, the optimal temperature for *B. bassiana* growth is 23–28ºC. The minimum temperature ranges from 5 to 10ºC, and maximum from 30 to 38ºC. At 50ºC, fungus spores die in 10 minutes. Application of *B. bassiana* biopreparation to control the Colorado beetle gave no effects at temperature almost 45ºC as fungus spores failed to germinate under such conditions (Sosnowska 2005). Average temperature of approximately 24ºC was sufficient to obtain high entomopathogenic effectiveness of *B. bassiana*; however, temperature decrease to 19ºC resulted in reduction of fungus effectiveness (Martin et al. 1999). It is a common knowledge that Boverol preparation retains its characteristics even under the conditions of high temperature and low humidity for the period of 3 months (Kreutz et al. 2001). According to the label, BoVeril preparation is active at soil temperature ranging from 12ºC to 26ºC. Under conditions of the present study, relatively low air temperatures during the treatments had probably no effect on biopreparation activity. Cool weather with relatively little sunlight is beneficial for survival of *B. bassiana* spores, thus the treatments should be generally carried out on cool and cloudy days (personal communication with the producer of biopreparation). Precipitation is an important factor decreasing biopreparation efficacy due to rinsing off from the surfaces of treated objects (for example, displayed bark beetle trap logs). Cold and rainy weather restrains insect swarming, and probably that is why pheromone trap efficiency observed in this study was relatively low. However, in all the years of observations, later in the vegetation season, air temperature raised to 21ºC and more, which was sufficient for *B. bassiana* spore germination. It is worth pointing out that the temperature of trap logs used in the trials was higher (temperature and humidity of tree bark and phloem are usually higher when compared to those of surrounding environment), thus it can be assumed that the conditions for germination of *B. bassiana* spores on treated logs were comparable to those determined as optimal for development of this fungus.

The results of the study of Bałazy (1962) carried out on *I. typographus* beetles treated with *B. bassiana* spore powder under field conditions, showed mortality of treated adult in galleries as a result of fungal infection. No fungus transmission was observed on *I. typographus* eggs or larvae. Very low transmission of the fungus from *I. typographus* parental generation toward young population was observed by Vaupel and Zimmermann (1996). These authors reported 23%–29% bark beetle mortality in *I. typographus* adults treated with Boverol and then placed onto the bark of fresh spruce bolts. Similar experiments carried out in the present study, showed no expected results. *B. bassiana* infected *I. typographus* adults (dusted with BoVeril dry formulation) not only stayed alive, but they normally went through all developmental stages in channelled out galleries with comparable parameters as those observed in the control. Only in one case (in 2013), *I. typographus* treated with dry formulation of Boverol did not establish galleries. Most probably, dusted bark beetles died due to the clogging of trachea with fungus spore powder. The differences between the effects of BoVeril and Boverol may be due to dissimilar formulation consistencies in the two biopreparations.

Spraying Norway spruce bolts with water suspension of Boverol and BoVeril performed both before and after *I. typographus* infestation also showed no expected results. Neither increased mortality nor decreased fecundity were observed, and in the galleries channelled out, there were found only individual adult specimens overgrown by *B. bassiana* mycelium. On the other hand, earlier attempts to control bark beetles with the fungus also showed no spectacular results. Cichońska and Świeżyńska (1993) observed very low (1.7%) proportion of bark beetles infected by *B. bassiana* when testing Norway spruce bolts treated before *I. typographus* infestation. Jakuš and Blaženec (2011), treated cut down trees that were infested by bark beetles and reported 29% fungal infection in pest population observed. On the other hand, however, the authors provided no clear information on either the size of investigated sample or calculation mode, hence it is difficult to judge the real effect of the treatment applied in their study.

In view of the present study, the only positive effect of biopreparation treatments was the reduction of the intensity of spruce mortality due to *I. typographus* infestation. In general, all through the whole area of the Forest District Jeleśnia (selected for the trials), there was observed a decreasing trend in the mortality of infested trees (Grodzki 2013). Nonetheless, in the forest divisions Koszarawa Bystra and Sopotnia Potok (showing similar extents of bark beetle infestations in the years preceding the trials), there was evidently observed impediment of spruce mortality rate in the region of bipreparation treatments. The difference was even more explicit, when there were compared the treated and control stands growing in comparable environmental conditions (similar a.s.l. and slope exposure), which showed analogous extents of spruce mortality in the years preceding the trials. The control stands were situated not far from the nature reserve Romanka excluded from forest protection activities, therefore, one could suppose that this area could be more threatened to *I. typographus* attacks. Nevertheless, both control and treated stands showed similar bark beetle infestation level before the trials, and after artificial introduction of *B. bassiana* biopreparation, there was observed a considerable decrease in spruce mortality in the stands treated compared to those untreated. Taking into consideration forest protection goals, positive effects of *B. bassiana* treatments combined with other forest protection methods (as done in this study in the area of the forest division Koszarawa Bystra) was obtained by Vakula et al. (2012). However, it seems that the results obtained so far have shown no coherent proof for the effectiveness of *B. bassiana* biopreparation in bark beetle control that could support promotion of using the fungus in the protection of Norway spruce stands against *I. typographus* and associated cambiophagous insects.

Sosnowska (2013) points out that using parasitic fungi in plant protection practice is often hardly effective given that the success of biological control relies upon still poorly understood mechanisms involved in relationships among the parasite, its host and environment. Nevertheless, under natural conditions, entomopathogenic fungi play an important role in regulating population numbers of numerous pests. Karg and Bałazy (2009) believe that forests enhance the development of fungi associated with insects. The authors emphasise that compared to agro-ecosystems, forests are more than two-fold richer in entomopathogenic fungi with high potential to decrease insect pest populations with no anthropogenic intervention.

When undertaking the trials presented in this study, it was assumed that the results obtained would allow to elaborate guidelines for forestry practice with regard to possibilities of application of the entomopathogenic fungus *B. bassiana* in forest protection, especially against cambiophagous insects in Norway spruce stands, where protection means are limited due to protective functions of forests. Although indirect effects of *B. bassiana* treatments were observed (less pest infested trees compared to the control), the results of 3-year study showed not sufficient enough evidence to recommend *B. bassiana* biopreparations for integration with other pest control methods used in Norway spruce stands susceptible to *I. typographus* and other insects associated with this species. The results obtained are important in view of the attempts made by the producers of *B. bassiana* biopreparations to introduce their products into pest control practice in Poland's forests. Based on the results of this study, it can only be pronounced that biopreparations based on *B. bassiana* continue to remain a 'promising prospect'. Further research under the conditions of forest environment is needed to better understand the relationships between the parasitic fungi, insect pests and host plants, as well as the effects of natural environment on efficacy of treatments with the use of entomopathogenic fungi. Only then, an appropriate strategy on the use of relevant biopreparations in control of populations of forest insect pests could be developed.

5. Conclusions

1. The results of 3-year-long study on the use of biopreparations with spores of the entomopathogenic fungus *Beauveria bassiana* under conditions of managed forests showed no satisfactory outcome in terms of control of bark beetle populations on Norway spruce.

2. *I. typographus* adults observed on Norway spruce log traps treated with *B. bassiana* biopreparation spray or dust (before and after infestation by bark beetles), as a general rule successfully completed their development or else were parasitised by hymenopterans (especially *Coeloides bostrychorum*), which also smoothly accomplished all developmental stages.

3. *I. typographus* adults captured in pheromone traps containing biopreparation dry formulation were contaminated with fungus spores; however, too much of powder on the body caused insect death owing to the clogging of trachea (not fungus entomopathogenic activity).

4. No traces of fungus transmission to *I. typographus* galleries was observed in treated and infested trees. Bark beetles overgrown by fungus mycelium were hardly ever found on Norway spruce log traps or adjacent infested standing trees.

5. Limited entomopathogenic effects of *B. bassiana* biopreparation on cambio- and xylophages overwintering in the litter were detected.

6. No *B. bassiana* biopreparation effects were observed as a result of direct contamination of *I. typographus* adults with the fungus either before or after establishing galleries. There was also not observed any influence of the treatment on bark beetle development and fecundity.

7. In view of the protection of forest against insect pests, indirect effects of *B. bassiana* treatments were observed. Mortality of infested spruces was decreased in the treated stands compared to those untreated. On the other hand, in all the stands, there were performed standard activities on forest protection against cambiophagous insects.

8. The method of control of *I. typographus* population based on *B. bassiana* treatments should be further tested, also in consideration of the effect on non-target insects associated with bark beetle populations. At this stage, there is no sound basis for recommendation of *B. bassiana* biopreparations for application in the protection of Norway spruce stands against *I. typographus* and related forest insect species.

Conflict of interest

None declared.

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References

Bałazy S. 1962. Obserwacje nad występowaniem niektórych grzybów owadobójczych z grupy *Fungi Imperfecti* na owadach leśnych. *Polskie Pismo Entomologiczne Seria B, Entomologia Stosowana* 2–3 (27–28): 149–164.

Bałazy S. 1966. Organizmy żywe jako regulatory liczebności populacji korników w drzewostanach świerkowych ze szczególnym uwzględnieniem owadobójczych grzybów. I. *Prace Komisji Nauk Rolniczych i Leśnych Poznańskiego Towarzystwa Przyjaciół Nauk* XXI, 1: 3–50.

Bałazy S. 1968. Analysis of bark beetle mortality in spruce forests in Poland. *Ekologia Polska (A)* 16(33): 657–687.

Bałazy S. 2012. Antagonistyczne oddziaływania pomiędzy bezkręgowcami a ich patogenami grzybowymi w żerowiskach kambio- i ksylofagów, in: A. Mazur (ed.) Ochrona lasu – wybrane problemy historyczne i współczesne. Wyd. Uniwersytetu Przyrodniczego w Poznaniu: 51–63. ISBN 978-83-7160-669-4.

Bałazy S. 2013. Organizmy entomopatogeniczne, in: W. Grodzki (ed.) Kornik drukarz *Ips typographus* (L.) i jego rola w ekosystemach leśnych. Centrum Informacyjne Lasów Państwowych, Warszawa: 46–49. ISBN 978-83-63895-08-2.

Bałazy S., Bargielski J., Ziółkowski G., Czerwińska C. 1967. Śmiertelność dorosłych chrząszczy kornika drukarza – *Ips typographus* (L.) (Col., Scolytidae) w żerowiskach i jej przyczyny. *Polskie Pismo Entomologiczne* 37(1): 201–205.

Cichońska A., Świeżyńska H. 1993. Próby zastosowania biopreparatów grzybowych do ograniczania liczebności populacji niektórych szkodliwych owadów leśnych [Trials of application of fungal biopreparations for reducing the population density of some forest pests]. *Prace Instytutu Badawczego Leśnictwa* 761–769: 137–144.

Głowacka B., Świeżyńska H. 1993. Grzyby owadobójcze występujące na owadach leśnych [Fungi occuring on forest insects]. *Prace Instytutu Badawczego Leśnictwa* 761–769: 117–136.

Grodzki W. 2007. Wykorzystanie pułapek feromonowych do monitoringu populacji kornika drukarza w wybranych parkach narodowych w Karpatach. *Prace Instytutu Badawczego Leśnictwa, Rozprawy i Monografie* 8: 1–127. ISBN 978-83-87647-69-8.

Grodzki W. 2010. The decline of Norway spruce *Picea abies* (L.) Karst. stands in Beskid Śląski and Żywiecki: theoretical concept and reality. *Beskydy* 3(1): 19–26.

Grodzki W. 2013. Zagrożenie lasów górskich i podgórskich, in: Krótkoterminowa prognoza występowania ważniejszych szkodników i chorób infekcyjnych drzew leśnych w Polsce w 2012 roku. *Instytut Badawczy Leśnictwa, Analizy i Raporty* 20: 124–137. ISBN 978-83-62830-16-9,

Jakuš R., Blaženec M. 2011. Treatment of bark beetle attacked trees with entomopathogenic fungus *Beauveria bassiana* (Balsamo) Vuillemin. *Folia Forestalia Polonica, Series A – Forestry*, 53(2): 150–155.

Karg J., Bałazy S. 2009. Wpływ struktury krajobrazu na występowanie agrofagów i ich antagonistów w uprawach rolniczych. *Progress in Plant Protection/Postępy w Ochronie Roślin* 49(3): 1015–1034.

Karpiński J.J. 1935. Przyczyny ograniczające rozmnażanie się korników drukarzy (*Ips typographus* L. i *Ips duplicatus* Sahlb.) w lesie pierwotnym. *Instytut Badawczy Lasów Państwowych Warszawa, Seria A Rozprawy i sprawozdania* 15: 1–65.

Kreutz J., Vaupel O., Zimmermann G. 2004a. Efficacy of *Beauveria bassiana* (Bals.) Vuill. against the spruce bark beetle, *Ips typographus* L., in the laboratory under various conditions. *Journal of Applied Entomology* 128(6): 384–389. DOI: 10.1111/j.1439-0418.2004.00813.384–389.

Kreutz J., Zimmermann G., Marohn H., Vaupel O., Mosbacher G. 2000. Preliminary investigations on the use of *Beauveria bassiana* (Bals.) Vuill. and other control methods against the bark beetle *Ips typographus* L. (Col., Scolytidae) in the field. *Bulletin OILB/SROP* 23(2): 167–173. ISBN 92-9067-119-X.

Kreutz J., Zimmermann G., Marohn H., Vaupel O., Mosbacher G. 2001. Freilandversuche zum Einsatz von *Beauveria bassiana* (Bals.) Vuill. zur biologischen Bekämpfung des Buchdruckers *Ips typographus* L. (Col., Scolytidae). *Mitteilungen der Deutschen Gesellschaft für allgemeine und angewandte Entomologi*e 13: 465–470.

Kreutz J., Zimmermann G., Vaupel O. 2004b. 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. **DOI:** 10.1080/788222844.

Kunca A., Vakula J., Leontovyč R., Gubka A. 2009. Využitie entomopatogénnej huby *Beauveria bassiana* v ochrane smreka, in: A. Kunca (ed.) Aktuálne problémy v ochrane lesa. Národné lesnícke centrum Zvolen: 91–97. ISBN 978-80-8093-081-3.

Landa Z., Kalista M., Křenová Z., Vojtěch O. 2008. Praktické využití entomopatogenní houby *Beauveria bassiana* proti lýkožroutu smrkovému *Ips typographus*, in: O. Vojtěch, P. Šustr (eds.) Ekologické metody ochrany lesa před podkorním hmyzem. Sborník referátů ze semináře, Sborníky z výzkumu na Šumavě - sešit 1. Správa NP a CHKO Šumava, Vimperk: 62–71.

Landa Z., Křenová Z., Vojtěch O. 2007. Využití houby *Beauveria bassiana* v ochraně proti lýkožroutu smrkovému. *Lesnická práce* 10: 14–15.

Translated by: Božena Kornatowska

Markova G. 2000. Pathogenicity of several entomogenous fungi to some of the most serious forest insect pests in Europe. *Bulletin OILB/SROP* 23(2): 231–239. ISBN 92-9067-119-X.

Markova G., Samshinyakova A. 1990. Laboratory studies on the effect of the entomopathogenic fungus *Beauveria bassiana* on larvae of *Lymantria dispar*. *Nauka za Gorata* 27(1): 81–84.

Martin P.A.W., Schroder R.F.W., Poprawski T.J., Lipa J.J., Sosnowska D., Hausvater E., Rasocha V. 1999. The effect of high temperatures on the susceptibility of the Colorado potato beetle (Coleoptera: Chrysomelidae) to *Beauveria bassiana* (Balsamo) Vuillemin in Poland, the Czech Republic and the United States. *Vědecké práce, Výzkumný ústav bramborářský Havlíčkův Brod*, 13: 69–77.

Onyśko Z., Starzyk J.R. 2011. Przestrzenne rozmieszczenie zimujących chrząszczy kornika drukarza (*I. typographus* L.) i kornika zrosłozębnego (*I. duplicatus* C. R. Sahlb.). *Sylwan* 155(1): 21–30.

Sierpińska A., Grodzki W. 2012. Badania nad wykorzystaniem *Bacillus thuringiensis* i entomopatogenicznych grzybów w ochronie lasu, in: I. Skrzecz, A. Sierpińska (eds.) Kierunki rozwoju patologii owadów w Polsce. Instytut Badawczy Leśnictwa, Sękocin Stary: 144–155. ISBN 978-83-62830-11-4.

Sosnowska D. 2005. Biopreparaty grzybowe w biologicznym zwalczaniu upraw szklarniowych i polowych. *Postępy Nauk Rolniczych* 5: 17–27.

Sosnowska D. 2013. Postępy w badaniach i wykorzystanie grzybów pasożytniczych w integrowanej ochronie roślin. *Progress in Plant Protection/Postępy w Ochronie Roślin* 53(4): 747–750.

Vakula J., Gubka A., Galko J., Varkonda Š. 2012. Aplikácia entomopatogénov do populácii škodcov s využitím feromónových lapačov, in: A. Kunca (ed.) Aktuálne problémy v ochrane lesa. Národné lesnícke centrum Zvolen: 92–96. ISBN 978-80-8093-161-2.

Vakula J., Varkonda Š., Galko J., Gubka A., Kunca A., Zúbrik M. 2010. Rozvoj sučasných technických možnosti pri štúdiu niektorých spôsobov aplikácie entomopatogénnej huby *Beauveria bassiana* v rámci biologických metód ochrany lesa, in: A. Kunca (ed.) Aktuálne problémy v ochrane lesa. Národné lesnícke centrum Zvolen: 64–68. ISBN 978-80-8093-108-7.

Vaupel O., Zimmermann G. 1996. Orientierende Versuche zur Kombination von Pheromonfallen mit dem insektenpathogenen Pilz *Beauveria bassiana* (Bals.) Vuill. gegen die Borkenkäferart *Ips typographus* L. *Anzeiger für Schädlingskunde, Pflanzenschutz, Umweltschutz* 69(8): 175–179.

Wegensteiner R. 1992. Untersuchungen zur Wirkung von *Beauveria*-Arten auf *Ips typographus* (Col., Scolytidae). *Bulletin OILB/SROP* 8(1-3): 104–106.

Wegensteiner R. 1996. Laboratory evaluation of *Beauveria bassiana* (Bals.) Vuill. against the bark beetle, *Ips typographus* (L.) (Coleoptera, Scolytidae). *Bulletin OILB/SROP* 19(9): 186–189.

Zimmermann G. 2007. Review on safety of the entomopathogenic fungi *Beauveria bassiana* and *Beauveria brongniartii*. *Biocontrol Science and Technology* 17: 553–596. DOI:10.1080/09583150701309006.

Authors' contribution

W.G. – research concept, preparation of methodology, fieldwork, data treatment, manuscript preparation. M.K. – fieldwork, modification of traps and ground photo-eclectors, data collection.