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Bioassays of Entomopathogenic Fungi against Xylophagous Insects in Bulgaria: Laboratory and Field Experiments

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Abstract: Six isolates of *Beauveria bassiana* (Bb) and one *Metarhizium anisopliae* (Ma) isolate were tested against adults of the European spruce bark beetle, *Ips typographus*, in laboratory assays. At a dosage of 1.5 x 10^6 conidia/cm2, mortality was significantly higher for treated *I. typographus* than for control individuals. Mortality rates of *I. typographus* reached 100% at four days post treatment with the isolates 619Ma, 638Bb and 639Bb. Field bioassays were then conducted using these three isolates in the Vitosha Mt., Bulgaria. Spruce logs were treated with conidial suspensions (10^6 conidia/cm^2). Three months later, bark was peeled from the logs and 1126 beetles belonging to ten coleopteran species (Curculionidae and Cerambycidae) were collected, identified and analysed for fungal pathogens. Analysis revealed natural occurrence of *B. bassiana*, *B. caledonica* and *Isaria farinosa*. Mortality rates of beetles collected from logs treated with 562Bb, 638Bb and 619Ma were 3.88%, 23.08% and 30.56%, respectively, and isolates 638Bb and 619Ma were significantly different from controls (P = 0.001). Isolate 562Bb was marginally significantly different from the control (P = 0.05). Results showed the potential to inoculate bark beetles with entomopathogenic fungi by treating spruce tree logs.

Key words: xylophages, entomopathogenic fungi, *Beauveria bassiana*, *Metarhizium anisopliae*, laboratory bioassays, field trial

Introduction

Xylophagous beetles of the families Curculionidae and Cerambicidae are usually secondary pests attacking weakened trees but they may become serious pests with a great importance for forest stands (especially coniferous) in the cases with insect population outbreaks. They have been studied in order to reveal factors reducing their population density including natural enemies – predators, parasitoids, nematodes and pathogens (Kenis et al. 2004). Possibilities for contact with pathogens are limited mainly by the hidden manner of their living. The majority of their life cycle stages occurs under the bark of host trees but infections caused by viral, protozoan and fungal pathogens have been registered (Weiser & Wegensteiner 1994, Wegensteiner & Weiser 1996, 2004, Händel et al. 2003, Sosnowska et al. 2004, Wegensteiner 2004, Takov et al. 2006, 2011, 2012, Draganova et al. 2007, 2010, Popa et al. 2012, Wegensteiner et al. 1996, 2015a,b). As naturally occurring mycoses are at low infection level in insect populations, introduction of inoculum of highly virulent strains of entomopathogenic fungi is considered to increase their prevalence.

Laboratory bioassays showed that bark beetles were susceptible to mycoses caused by the entomopathogenic fungi *Beauveria bassiana* (Balsamo-Crivelli) Vuillemin and *Metarhizium anisopliae* (Metschnikoff) Sorokin (Markova & Samsinakova 1990, Wegensteiner 1992, 1996, Markova 2000, Kreutz et al. 2004a,b, Draganova

et al. 2007, Sevim et al. 2010, Mudroncekova et al. 2013, Kocaçevik et al. 2016).

Contrary to expectation, field experiments were with varying success (Kreutz et al. 2001, 2004b, Dubois et al. 2004, Kunca et al. 2009, Vakula et al. 2010, Jakuš & Blaženec 2011, Grodzki & Kosibowicz 2015). Experiments conducted under natural conditions revealed some difficulties connected with application method used and conservation of propagules viability.

The aim of the study was to inoculate forest insect pests colonising *Picea abies* Karst. logs with *Beauveria bassiana* and *Metarhizium anisopliae* isolated from curculionids under natural conditions.

Materials and Methods

Fungal isolates

Six isolates of *B. bassiana* (433Bb, 434Bb, 562Bb, 563Bb, 638Bb and 639Bb) and one (619Ma) of *M. anisopliae* were used in the laboratory bioassays and three of them (562Bb, 638Bb and 619Ma) in experiments under natural conditions. They originated from dead adults of the following curculionids: *Ips typographus* Linnaeus (433Bb, 434Bb, 638Bb and 639Bb), *Hylurgops palliatus* Gyllenhal (562Bb and 563Bb) and *Tanymecus dilaticollis* Gyllenhal (619Ma). Isolates were obtained in pure cultures and characterised in our previous studies (Draganova et al. 2010, 2012). Pure cultures of the isolates were maintained in the collection of entomopathogenic fungi at the Entomology Department (Institute of Soil Science, Agrotechnologies and Plant Protection, Bulgaria).

Bioassays against adults of *Ips typographus* under laboratory conditions

Fungal isolates were cultured on SDAY (Sabouraud dextrose agar with yeast extract) slopes in tubes for 15 days at 25 ± 1°C. Obtained conidia were washed down with sterilised water containing 0.01% Twin 80 and suspensions were diluted to a concentration of 1 x 10^8 conidia/ml after determination of the concentration by enumeration of conidia in Bürker chamber. One ml of the conidial suspension of each isolate was dropped on filter-paper discs (diameter 9 cm) in Petri dishes.

Adults of *Ips typographus* used in the laboratory bioassays as test and control insects were collected from the bark of a fallen Norway spruce tree (*Picea abies*) colonised by a natural population of the pest in the Rila Mts. at 1500 m a.s.l. (42°04'08.3"N, 23°50'38.4"E). Test insects (by 30 individuals, in three repetitions) were contaminated with conidia of the fungal isolates by a contact with the previously treated filter-paper discs in Petri dishes (1.57 x 10^6 conidia/cm^2). Control insects were treated with sterilised water with 0.01% Tween 80. Insects were moved to untreated Petri dishes and fed on pine bark 24 h after the treatment. Experiments were carried out at 25 ± 2°C and 60 % RH for 7 days. Insect mortality was checked daily and each dead individual was removed and placed into a humid chamber for growth of the fungal pathogens. The results of the experiments were evaluated as percentages of cumulative daily mortality due to mycosis after correction with mortality in the control variant following Schneider-Orelli's formula (Püntener 1981). Virulence of each fungal isolate was estimated by values of the median lethal time (LT_{50}), calculated by probit analysis (Finney 1971). Statistical analyses were performed using the STATISTICA^R 6.0 software (Stat Soft Inc).

Experiment with fungal isolates against xylophages under natural conditions

One *M. anisopliae* and two *B. bassiana* isolates (619Ma, 562Bb and 638Bb) were used in the field trial after selection according to their virulence to adults of *I. typographus* in the laboratory experiments.

Necessary spore inoculum was produced in two-step cultivation (first in liquid medium and then on solid medium) under laboratory conditions. Propagative biomass (mainly blastospores) was obtained in three-days-old submerged cultures of the isolates after inoculation of Sabouraud dextrose liquid medium (50 ml per flask with 500 ml volume) with conidial suspensions (by 1 ml at concentration of 1 x 10^8 conidia/ml) and cultivation on shaker at 150 min^{-1} at 25°C. On the second step of the inoculum production 1 ml of the submerged cultures was spread on SDAY plates in Petri dishes (diam. 14 cm) and grown for 15 days at 25°C. Obtained conidia were scraped away and used to prepare suspensions (1 mg/ml) for field trials. Twin 80 (0.01%) was added to suspensions as a surfactant.

The field experiment was conducted in a spruce forest attacked by natural populations of *I. typographus* near to Aleko Hut (Vitosha Mt., 1720 m alt., 42°35'31.3"N, 23°17'56.6"E) on 04.07.2013. An alive spruce tree with height of 21.8 m and diameter at breast height of 35 cm was cut down. The first 10 m from the tree base was cut into 1 m long logs (with surface 0.775-1.334 m^2). One week later nine logs (in three groups, each one with three logs) were treated by spraying with conidial suspensions of the isolates 562Bb and 638Bb of *B. bassiana* and

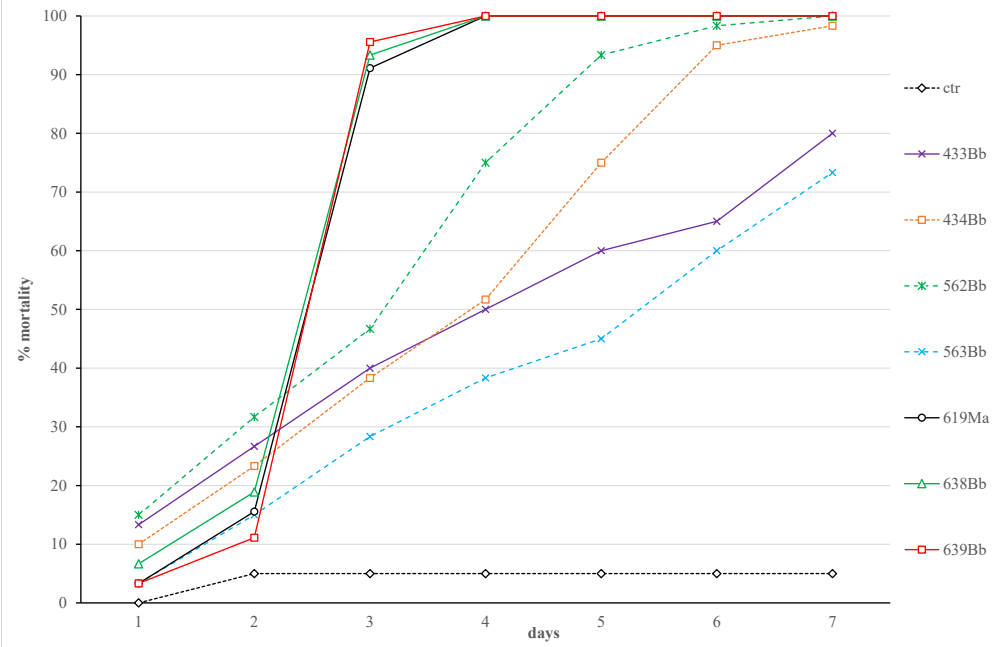

Fig. 1. Lethal effect (% cumulative daily mortality) of a control group (ctr) and groups with mycoses caused by fungal isolates 433Bb, 434Bb, 562Bb, 563Bb, 638Bb and 639Bb of *Beauveria bassiana* and 619Ma of *Metarhizium anisopliae* to adults of *Ips typographus* for 7 days. SE (Standard Errors): SE-ctr = ± 0.83%, SE-433Bb = ± 8.12%, P=0.0008, SE-434Bb = ± 13.03%, P=0.0065, SE-562Bb = ± 13.92%, P=0.0025, SE-563Bb = ± 8.40%, P=0.0086, SE-619Ma = ± 18.74%, P=0.0065, SE-638Bb = ± 18.14%, P=0.0047, SE-639Bb = ± 19.36%, P=0.0079

Table 1. Virulence of isolates of *Beauveria bassiana* and *Metarhizium anisopliae* to adults of *Ips typographus* using conidial suspensions (1 x 10^6 conidia/cm^2) in laboratory conditions. Letters indicate significant difference ($P < 0.05$) between virulence of the isolates

Fungal isolate, species	Median lethal time LT_{50} (days)			Regression coefficient
	Average values	Confidence intervals (P<0.05)		
		from	to	
433Bb *B.bassiana*	3.867[b]	3.505	4.255	3.1853 ± 0.2464
434Bb *B.bassiana*	3.370[b]	3.143	3.615	5.3431 ± 0.2856
562Bb *B.bassiana*	2.828[a]	2.571	3.112	5.4448 ± 0.3426
563Bb *B.bassiana*	4.709[c]	4.401	5.039	3.8252 ± 0.2178
619Ma *M. anisopliae*	2.901[a]	2.756	3.054	10.9257 ± 0.3542
638Bb *B.bassiana*	2.616[a]	2.431	2.816	8.6047 ± 0.3542
639Bb *B.bassiana*	2.870[a]	2.731	3.016	11.4045 ± 0.3542

619Ma of *M. anisopliae* at concentrations 100 mg/m^2 (10^6 conidia/cm^2). Control variant was sprayed with water and Twin 80 (0.01%). The field experiment was conducted on cool and foggy day, humidity was near 99 % and the temperature was < 20°C.

A week later the first observations for alive and dead insects on the treated logs were conducted. Colonisation of logs by insects was checked three months later. The bark of the treated and control logs was peeled and all insects were collected and analysed for fungal pathogens in the laboratory. Each dead insect after surface sterilisation was placed in a humid chamber for growth and sporulation of fungal pathogens. Small parts of cadavers with sporulated fungi were used to prepare microscope slides with lactophenol and aniline blue closed by nail varnish (after Humber 2005). Fungal pathogens were identified after Samson et al. (1988), Humber (2005) and Rehner et al. (2011) according to their morphological characters examined using a transmission interference microscope BX60 DIC Olympus equipped with digital camera and Cell B image capture software.

Chi-square Statistic was used to establish differences in mortality rates between the control and single treatments (Essi 1987).

Results

Laboratory experiments

Conducted laboratory bioassays showed that mortality caused by the examined fungal isolates to adults of *Ips typographus* was significantly higher when compared to control treatments ($P \leq 0.0086$; Fig. 1). The initial effect established on the 2nd day in the variants with isolates 433Bb, 434Bb and 562Bb was 26.67% ± 8.12, 23.33% ± 13.03 and 31.67% ± 13.92, respectively. Four days after the treatment with conidial suspensions of isolates 619Ma, 638Bb and 639Bb the mortality rates increased to 100% and to 75.00% ± 13.92 in the variant with isolate 562Bb. Mortality rates in the variants treated with isolates 563Bb and 434Bb were lower. On 7th day after the treatment they reached 73.33% ± 8.40 and 80.00% ± 8.12, respectively.

Isolates 638Bb, 562Bb, 639Bb and 619Ma were established to be the most virulent to adults of *I. typographus* with average values of the median lethal time 2.828 days, 2.616 days, 2.870 days and 2.901 days, respectively. Calculated values of LT_{50} for the fourth variants varied within narrow overlapped confidence intervals so they could not be differentiated by their virulence (Table 1). Isolates 434Bb and 433Bb were less virulent, followed by the isolate 563Bb (Fig. 2). The average values of the median lethal time and confidence intervals calculated at $P < 0.05$ for variants treated with isolates 433Bb and 434Bb were 3.867 (3.505-4.255) and 3.370 (3.143-3.615) days, respectively, and significant difference could not be proved between them. Isolate 563Bb was considered to be with lower virulence than all examined isolates (Fig. 2).

Field experiment

The experiment conducted under natural conditions showed that there were another insect species besides *I. typographus* that attacked *P. abies* trees in the selected location (Table 2). One thousand one hundred and twenty six larvae and adults of xylophagous insects belonging to 11 species: ten Coleopterans of the families Curculionidae [*Dendroctonus micans* (Kugel.), *Dryocoetes autographus* (Ratz.), *Hylastes cunicularius* (Erichson), *Hylobius abietis* L., *I. typographus*, *Pissodes pini* L., *Pissodes* sp.] and Cerambycidae (*Monochamus sutor* L., *Rhagium inquisitor* L., *Tetropium castaneum* L.) and one Hymenopteran from Siricidae (*Sirex juvencus* L.) were collected.

All Cerambycidae, *Pissodes* spp. and *Hylobius abietis* specimens mentioned in Table 2 were collected and studied as larvae. The only exception was one specimen of *H. abietis* in Variant 1 which was found as adult on the bark of the treated log and later died in the laboratory by a mycosis caused by *B. bassiana*. Bark beetles and *S. juvencus* were found as adults except for 52 larvae of *H. cunicularius* in Variant 2 infested with *B. bassiana*.

It was established that cerambycids were dominant in numbers (*M. sutor* – 470 specimens, *T. cas-*

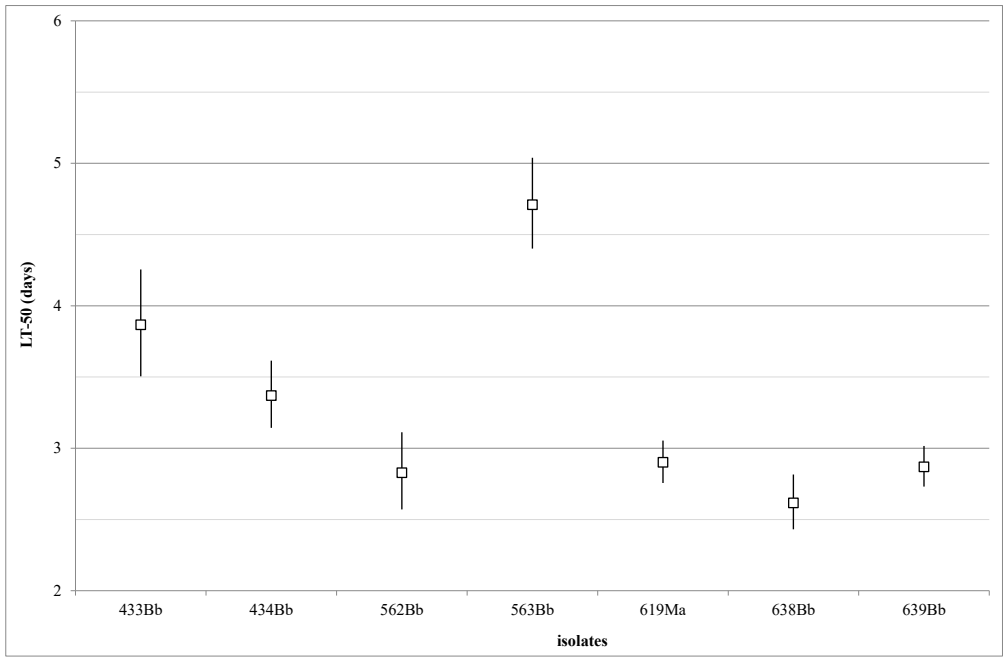

Fig. 2. Confidence intervals and average values of the median lethal time (LT_{50}) calculated for treatments with fungal isolates to adults of *Ips typographus*

Table 2. Insect pests colonising *Picea abies* logs treated with conidial suspensions of isolates of *Beauveria bassiana* and *Metarhizium anisopliae.* Abbreviations*:* Bb – individuals with mycosis caused by *B. bassiana*; Bc – individuals with mycosis caused by *B. caledonica*; If – individuals with mycosis caused by *Isaria farinose*; Ma – individuals with mycosis caused by *M. anisopliae*; UnR – individuals dead by unknown reasons

Insect species	Control	Variant 1 (562Bb)	Variant 2 (638Bb)	Variant 3 (619Ma)
1	2	3	4	5
Dendroctonus micans	0	0	0	0 1 *(Bb)
Dryocoetes autographus	0	Alive 1	0	Alive 4 2 A*(Bb)
Hylastes cunicularius	Alive 69 3 *(Bb) 1 *(Bc) 2 *(UnR)	Alive 50 2 *(Bb) 1 *(Bc) 2 *(UnR)	Alive 65 55 *(Bb) 2 *(If) 1 *(UnR)	Alive 3 2 *(Bb) 77 *(Ma)
Hylobius abietis	0	Alive 21 1 *(Bb)	Alive 10	Alive 12
Ips typographus	Alive 45 1 *(UnR)	0	Alive 1	Alive 1 1 *(Ma)
Monochamus sutor	Alive 201	Alive 129	Alive 34 1 *(Bb)	Alive 104 1 *(UnR)
Pissodes pini	Alive 11	0	0	Alive 3
Pissodes sp.	0	0	Alive 2	0
Rhagium inquisitor	0	Alive 16 1 *(UnR)	Alive 1 1 *(Bb)	Alive 8
Sirex juvencus	0	Alive 1	Alive 2 1 *(UnR)	0
Tetropium castaneum	0	Alive 5 2 *(UnR)	Alive 95 2 *(Bb)	Alive 64 4 *(Bb)

taneum – 172, *R. inquisitor* – 27) compared with curculionids with more species (7) but in smaller numbers (*H. cunicularius* – 335 specimens, *I. typographus* – 49, *H. abietis* – 44, *P. pini* – 14, etc.; Table 2).

The number of insects collected in the control variant was 336 specimens with repetition of only four species: *H. cunicularius*, *I. typographus, M. sutor* and *P. pini*. The average number of adults and larvae found per repetition in the treated variants was smaller than in the control: 77.33 specimens/repetition in the variant treated with 562Bb; 91.00 specimens/repetition in the variant with 638Bb and 95.67 specimens/repetition in the variant with 619Ma.

Adults and larvae of *H. cunicularius* were the most affected by mycoses after contact with logs treated with isolates 638Bb of *B. bassiana* (55 specimens) and 619Ma of *M. anisopliae* (77 specimens; Table 2). Only single numbers of bark beetles belonging to other species were infected by entomopathogenic fungi including *I. typographus.* In this study *Dendroctonus micans* was registered for the first time for Bulgaria as a host of *B. bassiana*. *Dendroctonus micans* is a notably dangerous pest on spruce trees (Six & Bracewell 2015) and this is its first record of natural occurring pathogens of this bark beetle in our country. That finding is also of interest from a faunistical point of view. *Dendroctonus micans* was reported for the first time in Bulgaria from other locality in Vitosha Mt. (Biolscheff 1934) but there were not any confirmed records of its finding in the country until now.

The recorded specimens of Cerambycidae were nearly 670 but only few of them were infected by entomopathogenic fungi (*M. sutor* – one specimen with *B. bassiana*, *T. castaneum* – six specimens with *B. bassiana*, *R. inquisitor* – one specimen with *B. bassiana*).

The established predators in the field experiment were presented by the larvae of Clerid beetles *Thanasimus formicarius* L. (two specimens) and *Thanasimus* sp. (17 specimens) with common preys conifer bark beetles. Only three individuals of *Thanasimus* sp. were registered in the control variant. All found predators were alive with the exception of one adult of *T. formicarius* which was with a mycosis caused by *B. bassiana*.

Microbiological analyses of the collected insects showed that *B. bassiana*, *B. caledonica* Bissett & Widden and *Isaria farinosa* (Holmsk.) Fr. ap-

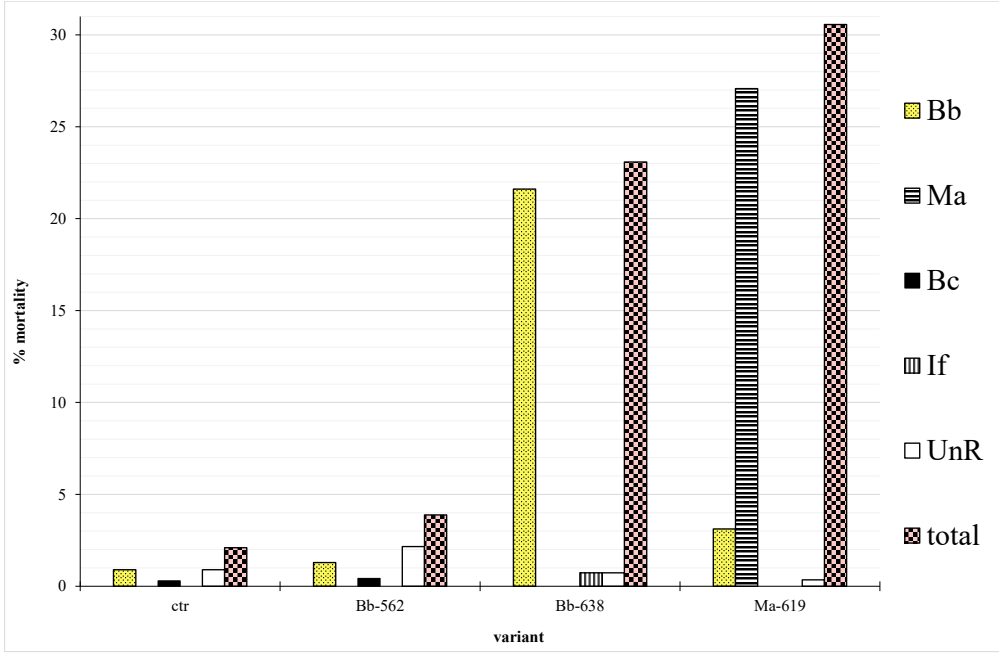

Fig. 3. Total mortality (%) and reasons for mortality of insect pests colonising spruce tree logs after treatment with conidia of isolates 562Bb and 638Bb of *Beauveria bassiana* and 619Ma and *Metarhizium anisopliae*

peared to be natural dwellers of the spruce tree wood colonised by xylophagous insects (Table 2, Fig. 3). The first two fungal species were found in the control variant, *B. caledonica* – on *H. cunicularius* in variant 1 (treated with conidial suspension of isolate 562Bb) as well, but *I. farinosa* – only in variant 2 (treated with conidial suspension of isolate 638Bb).

Calculated mortality rates in variants treated with conidial suspensions of isolates 562Bb and 638Bb of *B. bassiana* and 619Ma of *M. anisopliae* were 3.88%, 23.08% and 30.56%, respectively, but we supposed that real mortality rates were higher. It is possible that some insects after contact with treated logs and contamination with conidia flew away and escaped observation. It should be expected that infected insects in their movement would transmit entomopathogenic fungi to untreated susceptible hosts and would take part in autodissemination of the pathogens.

Significant differences in mortality rates were observed between the control and the treatments with isolates 638Bb and 619Ma at P = 0.001 (Chi-square (ctr-var.2) = 64.597 and Chi-square (ctr-var.3) = 96.491) whereas difference between the control and the treatment with isolate 562Bb were proved at P = 0.05 (Chi-square (ctr-var.1) = 1.57) (Fig. 3).

Discussion

The results from the laboratory experiments showed that adults of *I. typographus* were susceptible to mycoses caused by isolates 638Bb, 562Bb, 639Bb of *B. bassiana* and 619Ma of *M. anisopliae*. The average values of the median lethal time (2.616-2.901 days) manifested the observed mycoses as infections with a rapid lethal effect.

This is in correspondence with studies conducted by Wegensteiner (1996), Markova (2000), Kreutz et al. (2004a), Draganova et al. (2007), Sevim et al. (2010), Mudroncekova et al. (2013) on fungal isolates or commercial formulations of mycoinsecticides against different bark beetles. According to Wegensteiner (1996), *I. typographus* died rapidly (LT_{50}= 7.8 days) after contact with high-dose treatment ($7.5x10^7$ conidia/cm^2) of spruce-bark pieces with *B. bassiana*-spore powder formulation (the Czech preparation Boverol®) at 20°C. Very similar results were reported by Kreutz et al. (2004a) who studied efficacy of four *B. bassiana* isolates and Boverol® against *I. typographus* under various conditions (temperature, humidity and different substrates). The best results they achieved against insects dipped into a suspension of $1x10^7$ conidia/ml and transferred on spruce-bark pieces in Petri dishes at 25°C and 70% RH.

As the fungi *B. bassiana* and *M. anisopliae* are not host-specific, the results of our laboratory experiments might assist to achieve successful control not only of *I. typographus* but of other bark beetles after field application of the fungal pathogens.

Some of the species collected during the field experiment, such as *I. typographus, D. micans, H.*

cunicularius and *H. abietis*, are important pests on coniferous trees (in old-growth stands or young plantations) in Europe, the Mediterranean basin, Asia, North and Central America (Gregoire & Evans 2004, Lieutier et al. 2016). The most numerous insect found in this study, *M. sutor*, can be considered as a low aggressive pest but as a member of genus *Monochamus* it is also a potential vector of pine wood nematode, *Bursaphelenchus xylophilus* (Steiner & Buhrer) Nickle (see Evans et al. 2004). The domination of *M. sutor, T. castaneum* and *H. cunicularius* established in our experiment could be explained as follows: indicated longhorn beetles feed on the phloem but also deep in the wood where they have no competitors among the other common xylophages and *H. cunicularius* colonises the most humid parts of logs which are in contact with the ground.

The lower number of the adults and larvae of the pests found per repetition in the treated variants could be explained with some repellent action of the isolates of the entomopathogenic fungi against the insects colonising spruce tree logs. Similar repellent action of conidia of entomopathogenic fungi was observed in our previous laboratory experiments with the two-spotted spider mite (*Tetranychus urticae* Koch) and fungal isolates (Draganova & Simova 2010).

The finding of bark beetle predators during the field experiment could explain the death of some individuals of bark beetles marked as dead by unknown reasons in our study. According to Mills (1983) *T. formicarius* was recorded to feed on 27 bark beetle species belonging to 15 genera which infested coniferous and broad-leaved trees. Lieutier et al. (2016) considered that the clerids were found at high elevations and on the northern margins of the Mediterranean basin, but they were widely associated with *I. typographus* on spruce. On the other hand, predators and parasitoids could serve as vectors in spreading infections through transmission of fungal spores within bark galleries (Wegensteiner 2004).

The natural occurrence of *B. bassiana* in the control insects and *I. farinosa* in insects collected from the treated logs confirm the common distribution of both fungal pathogens. The detection of *B. caledonica* on *H. cunicularius* is the first record of this species in Bulgaria. Indeed we found few adults of *H. palliatus* in 2009 in Rila Mts. and *I. typographus* in 2010 in Vitosha Mt. with mycoses caused by *B. caledonica* (Draganova & Doychev, unpublished data). Two isolates of *B. caledonica* (644Bc and 706Bc) were isolated in pure cultures and the pathogen was identified at first according to its morphological characters and then the taxonomic status of the isolates was confirmed by PCR-based methods (Krumov, Georgieva & Draganova, unpublished data). The occurrence of *B. caledonica* is considered to be more seldom (Wegensteiner 2004, Glare et al. 2008).

The results of the conducted experiment confirmed the possibility to inoculate forest insect pests with conidia of entomopathogenic fungi by contact with treated spruce tree logs under natural conditions. They corresponded to some extent to results of the field studies of Kreutz et al. (2001, 2004b), Lavalée et al. (2005) and Jakuš & Blaženec (2011). In our opinion the occurred differences could be explained with differences in the applied methods, in virulence of different isolates of the entomopathogenic fungi, different environmental conditions, etc. Kreutz et al. (2001) used the combination of a pheromone trap and the biopreparation Boverol® against *I. typographus* to induce high beetle mortality in pest population. Contaminated with conidia bark beetles left the trap and carried the pathogen into the pest population. Later Kreutz et al. (2004b) examined in field cage experiments the horizontal transmission of *B. bassiana* (Boverol®) between beetles of *I. typographus* and dissemination of the fungus within a bark beetle population. They found that the rate of mycosis was 78% which was significantly higher than in the control. Lavalée et al. (2005) reported about a field experiment with encouraging results about application of *B. bassiana* as a potential control method against another bark beetle: 98%-100% cumulative mortality within four days in treated *T. piniperda*. The same *B. bassiana* preparation Boverol® as in the experiments of Kreutz et al. (2001, 2004b) with modified pheromone traps was used by Vakula et al. (2010) but the same effect under natural conditions was not obtained. The results of the experiment with *B. bassiana* against *I. typographus* carried out by Jakuš & Blaženec (2011) in the area of the Spišská Magura Mountains were very similar to our results of the outdoor experiment. They succeeded to infect about 28.75% of *I. typographus* individuals in the treated stems and this was significantly higher than in the control variant.

Unlike them, Grodzki & Kosibowicz (2015), after a three-year study, concluded that there was not sufficient evidence to recommend *B. bassiana* biopreparations for application in Norway spruce stands for control of *I. typographus* and other insects associated with this species.

Positive results of several field experiments, benefits and advantages of assisted autodissemination of entomopathogenic fungi over their mass ap-

plication described by Baverstock et al. (2010), considerations of Popa et al. (2012) and the results received in this study confirm the potential of the entomopathogenic fungi to control bark beetles and further research under the conditions of forest environment should be implemented.

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