Списък на естествено срещащи се гъбни патогени, заразяващи корояди и пеперуди – вредители в горите от България

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List of natural fungal pathogens infecting bark beetles and lepidopteran forest pests from Bulgaria

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Резюме: Представена е информация за установените гъбни инфекции в различни видове вредни насекоми – корояди и пеперуди от България. В 12 вида корояди и 10 вида пеперуди са идентифицирани общо 18 вида ентомопатогенни гъби и многобройни изолати, принадлежащи към разредите Hypocreales, Eurotiales и Entomophthorales за шейсетгодишен период. Обобщени са резултатите от лабораторни и полеви опити, проведени с част от намерените гъбни изолати срещу корояди и гъботворката (*Lymantria dispar).*

Ключови думи: ентомопатогенни гъби, корояди, пеперуди

Abstract: Information about fungal infections detected in different bark beetles and lepidopteran forest pests in Bulgaria is presented. Eighteen species of entomopathogenic fungal species and numerous isolates of orders Hypocreales, Eurotiales и Entomophthorales have been reported from 12 bark beetles and 10 moths within a sixty years period. The results from conduct-

https://doi.org/10.33919/ansd.19.1.10

ed laboratory and field bioassays with several isolates of entomopathogenic fungi against bark beetles and the gypsy moth were summarized.

Keywords: entomopathogenic fungi, bark beetles, lepidopteran pests

Introduction

Insect pests are a major cause of economic and aesthetic loss in forestry systems and are of particular concern as habitats become more fragmented, land use pressures increase, and climate change impacts forest ecosystems. Chemical pest controls cause additional concerns about effects on non-target organisms in treated areas and in the larger watersheds.

The most significant pests of European forests are species in the orders Coleoptera and Lepidoptera [Day & Leather, 1997]. In the coniferous forests of Bulgaria, bark beetles (Coleoptera: Curculionidae) and the pine processionary moth *Thaumetopoea pityocampa* (Denis & Schiffermüller), are of greatest concern. Important pests of broadleaf forests include moths from the families Erebidae (*Lymantria dispar* L., *Euproctis chrysorrhoea* L.), Notodontidae (*Thaumetopoea processionea* L.), Tortricidae and Geometridae [Zaemdzhikova et al., 2019]. Forestry administrations are committed to reducing the use of pesticides in the environment and to emphasizing the use of biological control agents. Therefore the research on the natural enemies of these insect pests including fungal pathogens is of great importance.

The aim of this study is to present information about fungal infections found in different bark beetles and lepidopteran forest pest species in Bulgaria.

1. Entomopathogenic fungi of bark beetles

Entomopathogenic fungi which attack bark beetles belong to the division Ascomycota, order Hypocreales and Eurotiales. They infect their hosts through the integument. The fungus proliferates throughout the insect and causes mortality of its host. Host specificity of entomopathogenic fungi varies. While some species are very host specific, others have a broad host range [Wegensteiner et al., 2015].

There are many reports for the occurrence of entomopathogenic fungi in bark beetles [Wegensteiner, 2004; Wegensteiner et al., 2015]. The first publications about fungal pathogens in bark beetles were from Great Britain, Poland, France and the Netherlands [Wegensteiner et al., 2015]. Petch [1932] reported *Beauveria bassiana* (Balsamo) Vuillemin from *Hylastes ater* (Paykull) in Great Britain and Karpinski [1935], Siemaszko [1939] discovered it in Poland. Since then the number of publications dealing with entomopathogenic fungi in bark beetles increased and at least 13 fungal species from 15 bark beetle species were reported by different authors [Wegensteiner et al., 2015]. Moreover entomopathogenic fungi were evaluated as bark beetles control agents. The most studied species was *B. bassiana*. Various bioassays have been conducted against different bark beetle species. First Doane [1959] reported high mortality of *Scolytus multistriatus* (Marsham) caused by *B. bassiana* in larvae, pupae and adults of the host. Later Novak and Samsinakova [1962] showed that *B. bassiana* was highly pathogenic to *Ips typographus* L. Other fungi such as *Metharizium anisopliae* (Metschn.) Sorokin, *M. brunneum* Petch and *Isaria fumosorosea* Wize were also tested against different bark beetles [Wegensteiner et al., 2015]. Commercial isolates of *B. bassiana* were also developed and evaluated [Castrillo et al., 2011].

In Bulgaria the investigations on bark beetles pathogens started in 2005 when Takov et al. [2006] detected *B. bassiana* in *I. typographus*. It was established in 2005 in 3 localities in Vitosha Mt. and its prevalence was very low (0.7%) [Takov et al., 2006, 2019]. In the same host were observed also *B. caledonica* [Draganova et al., 2017] and *Fusarium* sp. [Draganova, personal communication]. *B. bassiana* was found in *Ips sexdentatus* (Börner) [Takov et al., 2007; Draganova et al., 2010], *Dryocoetes autographus* (Ratzeburg) [Draganova et al., 2010] and *Hyllurgops palliatus* (Gyllenhal) [Takov et al., 2011, 2012] [Table 1]. Additionally Draganova et al. [2010] isolated *B. brongniartii* from *I. typographus* and *Isaria farinosa* from *I. sexdentatus*. The authors revealed totally 3 fungal species in 6 bark beetle species collected in 4 mountains – Vitosha, Rila, Maleshevska and Rhodopes. The most frequently found fungus of bark beetles in Bulgaria was *B. bassiana* followed by *B. caledonica* and *B. brongiartii* [Table 1 and Table 2].

Table 1. Data of insect hosts and their natural entomopathogenic fungi reported from Bulgaria

Insects	Host plant	*Localities	Fungal pathogens	Reference
Coleoptera, Curculionidae, Scolytinae				
Dendroctonus micans (Kugelann)	*Picea abies*	1	*Beauveria bassiana*	Draganova et al., 2017
Dryocoetes autographus (Ratzeburg)	*P. abies*	3	*Beauveria bassiana*	Draganova et al., 2010
		1	*Beauveria bassiana*	Draganova et al., 2017
Hylastes cunicularius (Erichson)	*P. abies*	1	*Beauveria bassiana* *Beauveria caledonica* *Isaria farinosa*	Draganova et al., 2017
Hylurgops palliatus (Gyllenhal)	*Pinus sylvestris*	45	*Beauveria bassiana*	Takov et al., 2011
	Picea abies	38	*Beauveria bassiana* *Beauveria brongniartii*	Takov et al., 2012
	Pinus sylvestris	45	*Beauveria caledonica*	Draganova et al., 2017
Ips acuminatus (Gyllenhal)	*P. sylvestris*	16	*Isaria farinosa* *Aspergillus* sp.	Takov et al., 2012
	Pinus nigra	52	*Beauveria bassiana* *Isaria farinosa* *Fusarium* sp.	unpublished data
Ips sexdentatus (Börner)	*Pinus sylvestris*	51	*Beauveria bassiana*	Takov et al., 2007
		49	*Beauveria bassiana* *Isaria farinosa*	Takov et al., 2012
		50	*Beauveria bassiana* *Isaria farinosa*	Draganova et al., 2010
	P. sylvestris	49	*Beauveria brongniartii*	unpublished data
Ips typographus (L.)	*Picea abies*	3	*Beauveria bassiana*	Takov et al., 2006
		4	*Beauveria bassiana* *Beauveria brongniartii*	Draganova et al., 2010
			Fusarium sp.	unpublished data
			Beauveria caledonica	Draganova et al., 2017
		2	*Beauveria bassiana*	Takov et al., 2007
		4	*Aspergillus* sp.	Takov et al., 2012
Orthotomicus erosus (Wollaston)	*Pinus sylvestris*	48	*Beauveria bassiana*	Takov et al., 2012
Orthotomicus longicollis (Gyllenhal)	*P. sylvestris*	49	*Beauveria bassiana*	Takov et al., 2012
Taphrorychus villifrons (Dufour)	*Fagus sylvatica*	5	*Beauveria bassiana*	Takov et al., 2012

Insects	Host plant	*Localities	Fungal pathogens	Reference
Tomicus minor (Hartig)	*P. nigra*	8	*Beauveria bassiana*	unpublished data
Tomicus piniperda (L.)	*P. sylvestris*	47	*Beauveria bassiana* *Aspergillus* sp.	Takov et al., 2012
Lepidoptera				
Catocala nymphagoga (Esper, 1787)	*Quercus* spp.	13	*Entomophaga aulicae* *Beauveria bassiana* *Isaria farinosa Lecanicillium* sp.	Georgieva et al., 2014
		14, 53	*Tarichium dissolvens* *Conidiobolus* sp.	
		25	*Beauveria bassiana* *Beauveria* sp.	
		15	*Beauveria* sp. *Fusarium* sp.	
Erannis defoliaria (Clerck)	*Quercus* spp.	28	*Aspergillus niger* *Beauveria bassiana*	Draganova et al., 2013
Eriogaster lanestris (L.)	*Crataegus sp.*	33	*Beauveria bassiana* *Fusarium* sp.	unpublished data
Euproctis chrysorrhoea (L.)	*Quercus* spp.	7, 9, 10, 12, 17, 18, 26, 27, 36, 37, 39, 40, 41, 43, 44, 46	*Entomophaga aulicae*	Pilaska et al., 2001
	Q. frainetto	35	*Entomophaga aulicae*	Pilaska et al., 2018
Leucoma salicis (L.)	*Populus x euramericana*	42	*Beauveria bassiana*	Markova and Georgiev, 1992
Lymantria dispar (L.)	*Quercus* spp.	23, 24	*Beauveria bassiana* *Beauveria globulifera* *Isaria farinosa*	Panajotov et al., 1960
	Quercus frainetto, *Q. cerris*	19, 20, 21	*Aspergillus flavus* *Aspergillus* sp. *Fusarium* sp. *Mucor globosus* *Mucor mucedo* *Penicillium frequentans* *Scopulariopsis brevicaulis*	Mirchev, 2004
	Quercus spp.	28	*Beauveria bassiana*	Draganova et al., 2011
	Quercus spp.	28, 29, 32	*Beauveria bassiana* *Aspergillus* sp.	Draganova et al., 2013
		31, 33, 34	*Beauveria bassiana* *Aspergillus* sp.	unpublished data

Insects	Host plant	*Localities	Fungal pathogens	Reference
Malacosoma neustria (L.)	*Quercus* spp.	32	*Beauveria bassiana* *Fusarium* sp.	Draganova et al., 2013
		30	*Beauveria bassiana* *Fusarium* sp.	unpublished data
Thaumetopoea pityocampa (Denis & Schiffermüller)	*Pinus nigra*	6	*Beauveria bassiana* *Aspergillus* sp. *Fusarium* sp.	Draganova et al., 2013
Thaumetopoea solitaria (Freyer)	*Pistacia terebinthus*	31	*Beauveria bassiana*	Mirchev et al., 2012
Tortrix viridana (L.)	*Quercus* spp.	11, 22, 28	*Beauveria bassiana* *Aspergillus* sp. *Fusarium* sp.	Draganova et al., 2013

*Localities. **Vitosha Mt.:** 1 – Aleko Hut, 2 – Artista Hut, 3 – Bistrishko branishte Biosphere Reserve, 4 – Zlatni mostove Place**; Lyulin Mt.:** 5 – St. St. Cyril and Methodius Monastery; **Sredna gora Mt.:** 6 – Banya Vill., 7 – Babek Vill., 8 – Hisarya, 9 – Kavakliyka Vill., 10 – Koprinka Vill., 11 – Pobit kamak Vill., 12 – Turiya Vill.; **Balkan range:** 13 – Elovitsa Vill., 14 – Skravena Vill., 15 – Ravna gora Vill., 16 – Gabrovo, 17 – Kalofer, 18 – Kazanlak, 19 – Kosta Perchevo Vill., 20 – Makresh Vill., 21 – Mramoren Vill., 22 – Plakovo Vill.; **Strandzha Mt.:** 23 – Bosna Vill., 24 – Zvezdets Vill., 25 – Indzhe Voyvoda Vill.; **Sakar Mt.:** 26 – Glavan Vill., 27 – Kostur Vill.; **Eastern Rhodopes Mts.:** 28 – Gnyazdovo Vill., 29 – Gugutka Vill., 30 – Huhla Vill., 31 – Ivaylovgrad, 32 – Kamenets Vill., 33 – Karamfil Vill., 34 – Silen Vill, 35 – Zhenda Vill.; **Western Rhodopes Mts.:** 36 – Asenovgrad, 37 – Bachkovo Vill., 38 – Beglika Place, 39 – Byala cherkva Vill., 40 – Iskra Vill., 41 – Parvomay, 42 – Pazardzhik, 43 – Perushtitsa, 44 – Ruen Vill., 45 – Yundola Vill., 46 – Zhalt kamak Vill.; **Rila Mt.:** 47 – Yakoruda; **Maleshevska planina Mt.:** 48 – Mikrevo Vill., 49 – Nikudin Vill., 50 – Razdol Vill., 51 – Tsaparevo Vill.; **Pirin Mt.:** 52 – Dobrinishte Vill.; **Danubian Plain:** 53 – Slavyanovo Vill.

Table 2. List of established fungal species and their insect hosts

Entomopathogenic fungi	**Insect hosts**	
	Bark beetles	**Moths**
Aspergillus flavus Link		*Lymantria dispar*
Aspergillus niger Tiegh.		*Erannis defoliaria*
Aspergillus sp.	*Ips acuminatus* *Ips typographus* *Tomicus piniperda*	*Erannis defoliaria* *Lymantria dispar* *Thaumetopoea pityo-campa* *Tortrix viridana*
Beauveria bassiana (Bals.-Criv.) Vuill.	*Dendroctonus micans* *Dryocoetes autographus* *Hylastes cunicularius* *Hylurgops palliates* *Ips acuminatus* *Ips sexdentatus* *Ips typographus* *Orthotomicus erosus* *Taphrorychus villifrons* *Tomicus minor* *Tomicus piniperda*	*Catocala nymphagoga* *Erannis defoliaria* *Eriogaster lanestris* *Euproctis chrysorrhoea* *Leucoma salicis* *Lymantria dispar* *Malacosoma neustria* *Thaumetopoea pityo-campa* *Thaumetopoea solitaria* *Tortrix viridana*
Beauveria brongniartii (Sacc.) Petch	*Hylurgops palliates* *Ips sexdentatus* *Ips typographus*	
Beauveria globulifera (Speg.) F. Picard		*Lymantria dispar*
Beauveria caledonica Bissett & Widden	*Hylastes cunicularius* *Hylurgops palliatus* *Ips typographus*	
Beauveria sp.		*Catocala nymphagoga*
Conidiobolus sp.		*Catocala nymphagoga*
Entomophaga aulicae (E. Reichardt) Humber		*Catocala nymphagoga* *Euproctis chrysorrhoea*
Fusarium sp.	*Ips acuminatus* *Ips typographus*	*Eriogaster lanestris* *Lymantria dispar* *Malacosoma neustria* *Thaumetopoea pityo-campa* *Tortrix viridana*
Isaria farinosa (Holmsk.) Fr.	*Hylastes cunicularius* *Ips acuminatus* *Ips sexdentatus*	*Catocala nymphagoga* *Lymantria dispar*
Lecanicillium sp.		*Catocala nymphagoga*
Mucor globosus A. Fisch.		*Lymantria dispar*
Mucor mucedo (Tode) Spreng.		*Lymantria dispar*
Penicillium frequentans Westling		*Lymantria dispar*
Scopulariopsis brevicaulis (Sacc.) Bainier		*Lymantria dispar*
Tarichium dissolvens Vosseler		*Catocala nymphagoga*

Laboratory experiments with fungal species against bark beetles

In 2000 Markova [2000] performed laboratory experiments with *Beauveria bassiana*, *Verticillium lecanii* (Zimm.) Viégas, *Isaria farinosa* and *Metarhizium anisopliae* against *Ips typographus* and showed that the bark beetle was susceptible to these fungi.

Later Draganova et al. [2007] also conducted laboratory bioassays with conidial suspensions of *B. bassiana* and *Isaria farinosa* isolates against *I. sexdentatus* and *I. acuminatus*. They established that 3 different isolates of *B. bassiana* caused the highest lethal effect to adults of *Ips sexdentatus* – between 89.33 and 96.67%. The adults of *I. sexdetnatus* were more susceptible to the isolates of *B. bassiana* than to these of *I. farinosa*.The results from bioassays with adults of *I. acuminatus* revealed that the host was not susceptible to *I. farinosa*.

In other research Draganova et al. [2017] performed laboratory bioassays using as test and control insects adults of *Ips typographus*. Conducted laboratory bioassays showed that mortality caused by the examined fungal isolates to adults of *I. typographus* was significantly higher when compared to control treatments. Initial effect established on the second day in the variants with 3 isolates was 26.67% ± 8.12, 23.33% ± 13.03 and 31.67% ± 13.92, respectively. Four days after the treatment with conidial suspensions of one isolate of *M. anisopliae*, and two from *B. bassiana* the mortality rates increased to 100% and to 75.00% ± 13.92, respectively. Mortality rates in the variants treated with isolates of *B. bassiana* were lower.

Field experiments with fungal species against bark beetles

Draganova et al. [2017] conducted the first field experiment under natural conditions in Bulgaria with bark beetles fungal pathogens. Totally, larvae and adults of insects belonging to 11 species were examined. The results showed that adults and larvae of *Hylastes cunicularius* were the most affected by mycoses after contact with Norway spruce logs treated with one isolate of *B. bassiana* (55 specimens) and one of *M. anisopliae* (77 specimens). Only single numbers of bark beetles belonging to other species were infected by entomopathogenic fungi including *Ips typographus.* In this study for the first time for Bulgaria *Dendroctonus micans* was registered as a host of *B. bassiana* and natural infections caused by *B. bassiana, B. caledonica, Isaria farinosa* in *Hylastes cunicularius*, *Dendroctonus micans* and *Dryocoetes autographus* [Table 1, Table 2].

2. Entomopathogenic fungi of lepidopteran forest pests

The study of fungal pathogens of leidopteran pest species was provoked in order to improve the existent strategies for their control. Entomopathogenic fungi infecting insects of order Lepidopetra belong to the division Ascomycota, order Hypocreales, Eurotiales and to the division Entomophthoromycota, order Entomophthorales.

In Bulgaria, the first report of entomopathogenic fungi in lepidopterans was published by Panajotov et al. [1960]. The authors recorded *B. bassiana*, *B. globulifera* and *I. farinosa* in larvae of the gypsy moth (*Lymantria dispar*). Markova and Georgiev [1992] reported *B. bassiana* in dead larvae of *Leucoma salicis.* Later Mirchev [2004] found *Scopulariopsis brevicaulis*, *Aspergillus flavus*, *Penicillium frequentans, Mucor mucedo*, *M. globosus*, *Aspergillus* sp. and *Fusarium* sp. which were the cause of 24.5% of mortality of *L. dispar* pupae. Draganova et al. [2011] confirmed the presence of *B. bassiana* in *L. dispar* larvae and and Mirchev et al. [2012] found it for the first time in two new hosts – *Thaumetopoea pityocampa* and *T. solitaria*. Later Draganova et al. [2013] established *B. bassiana* in 6 lepidopteran species – *T. pityocampa*, *Lymantria dispar*, *Malacosoma neustria*, *Tortrix viridana*, *Erannis defoliaria* and *Melitaea didyma* (Esper). The most affected by this pathogen were the larvae of *T. pityocampa*. The fungus was detected in 66.7% of all dead pine processionary moth larvae. This fungus caused mortality also in 7.2% of all *L. dispar*, 6.9% of *Malacosoma neustria*, 5.1% of *T. viridana* and 37.5% of *Erannis defoliaria* collected individuals. The mycosis was observed mainly in larvae, rarely in pupae. Besides *B. bassiana* these authors recorded more fungal pathogens – *Aspergillus niger* in *E. defoliaria, Aspergilus* sp. in *T. pityocampa, L. dispar* and *T. viridana*, and *Fusarium* sp. in *T. pityocampa, M. neustria* and *T. viridana* [Table 2]. *B. bassiana* and *Fusarium* sp were detected also in *Eriogaster lanestris* [Draganova, personal communications] [Table 1 and Table 2]. Georgieva et al. [2014] revealed 7 fungals species in another host, *Catocala nymphagoga* (Esper, 1787)[Table 1 and Table2].

Using seven different isolates of *B. bassiana* and one of *Metharisium anisopliae* Draganova et al. [2010] studied the susceptibility of *L. dispar* larvae to these fungi. The results of the conducted bioassays showed that these caterpillars were tolerant to all tested isolates.

The first fungus of order Entomophthorales recorded in lepidopterans from Bulgaria was *Entomophaga aulicae* [Pilarska et al., 2001]. It was observed in 2000 in high density of populations of the brown tail moth, *Euproctis chrysorrhoea* collected in Balkan range, Sakar, Sredna gora and Rhodopes Mountains. The authors established the pathogen in 16 out of 72 sites with brown tail moth infestation. *E. aulicae* was the main factor reducing *E. chrysorrhoea* population density. *E. aulicae* was recorded again in 2016 in a brown tail moth population in the region of Asenovgrad [Pilarska et al., 2018] [Table 1 and Table 2].

In order to improve the existing biological control of *Lymantria dispar* in 1999 in the region of Karlovo, a successful introduction of the entomopathogenic fungus *Entomophaga maimaiga* Humber, Shimazu & R.S. Soper was conducted in a gypsy moth population. In 2005 strong epizootics caused by this fungus were detected in different areas of Bulgaria. In the next years several new epizootics occurred and suppressed some strong outbreaks of the pest. As a result over the past 20 years, almost no insecticides have been used to control *L. dispar* [Pilarska et al., 2016].

In 2016 a successful release of another entomophthorous fungus, *E. aulicae* for control of *Euproctis chrysorrhoea* was performed. It was conducted in a healthy brown tail moth population near Kardzhali and larval mortality of 19% has been established subsequently [Pilarska et al., 2018].

Conclusion

During a sixty years period numerous isolates of 18 entomopathogenic fungal species and representatives of 11 genera from orders Hypocreales, Entomophthorales and Eurotiales were reported from 12 bark beetle and 10 lepidopteran species collected in 53 localities in Bulgaria. Several laboratory and field bioassays with entomopathogenic fungi were conducted and introductions of two entomophthoralean fungi were performed. In order to improve the biological control of forest pest insects the research on the entomopathogenic fungi should continue and should be intensified.

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