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Naturally-Occurring Entomopathogenic Fungi on Three Bark Beetle Species (Coleoptera: Curculionidae) in Bulgaria

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SUMMARY

Bark beetles (Coleoptera: Curculionidae: Scolytinae) belong to one of the most damaging groups of forest insects and the activity of their natural enemies – pathogens, parasitoids, parasites or predators suppressing their population density, is of great importance.

Biodiversity of entomopathogenic fungi on bark beetles in Bulgaria has been investigated sporadically. The aim of this preliminary study was to find, identify and study morphological characteristics of fungal entomopathogens naturally-occurring in populations of three curculionid species – *Ips sexdentatus* Boern, *Ips typographus* (L.) and *Dryocoetes autographus* (Ratz.).

Dead pest adults were found under the bark of *Pinus sylvestris* and *Picea abies* trees collected from forests in the Maleshevska and Vitosha Mountains. Fungal pathogens were isolated into pure cultures on SDAY (Sabouraud dextrose agar with yeast extract) and were identified based on morphological characteristics both on the host and in a culture. Morphological characteristics of the isolates were studied by phenotypic methods.

The fungal isolates obtained from dead adults of *Ips sexdentatus, Ips typographus* and *D. autographus* were found to belong to the species *Beauveria bassiana* (Bals. – Criv.) Vuillemin, *Beauveria brongniartii* (Saccardo) Petch and *Isaria farinosa* (Holmsk.) Fries (anamorph Ascomycota, Sordariomycetes: Hypocreales, Cordycipitaceae). Morphological traits of the isolates are described.

Keywords: Bark beetles; *Ips sexdentatus; Ips typographus; Dryocoetes autographus;* Entomopathogenic fungi; *Beauveria bassiana; Beauveria brongniartii; Isaria farinosa;* Morphological characteristics; Natural occurrence

INTRODUCTION

Ips typographus (L.) and *Ips acuminatus* Gyll. are some of the most damaging bark beetles (Coleoptera: Curculionidae: Scolytinae) on living trees in Europe according to Gregoire and Evans (2004).

In Bulgaria, about 300 ha of spruce stands in the forest reserve Bistrishko branishte (Vitosha Mountain) were destroyed some years ago due to damages caused by *Ips typographus* (Georgiev, 2006). The activity of their various natural enemies – pathogens, parasitoids, parasites or predators suppressing their population density is of great importance.

While *Beauveria bassiana* (Bals.-Criv.) Vuillemin has been reported as a fungal pathogen frequently occurring in populations of bark beetles, the species *Isaria fumosorosea* Wize (major synonym *Paecilomyces fumosoroseus* (Wize) Brown and Smith), *Isaria farinosa* (Holmsk.) Fries (major synonym *Paecilomyces farinosus* (Holmsk. ex Gray) Brown and Smith), *Verticillium lecanii* (Zimm.) Viegas and *Metarhizium anisopliae* (Metsch.) Sorokin have been mentioned only in a few reports (Landa et al., 2001; Jankevica, 2004; Sosnowska et al., 2004).

Wegensteiner (2004), reviewing records about pathogens of bark beetles, pointed out that there was insufficient knowledge of the activity of pathogens on bark beetles worldwide in spite of a presumed potential of pathogens in controlling bark beetle populations. According to him, a number of investigations have shown the occurrence of fungi in bark beetle field populations. Several fungal species were most frequently mentioned, including *B. bassiana* and *Isaria* sp. (synonyms *Spicaria*, *Paecilomyces*).

Biodiversity of entomopathogenic fungi on bark beetles in Bulgaria has been sporadically investigated. An isolate obtained from a dead adult of *Ips sexdentatus* Boer., collected from a natural environment, has been identified by Draganova et al. (2007) as *B. bassiana*.

The aim of this study was to find, identify and study the morphological characteristics of fungal entomopathogens occurring naturally in populations of three scolytid species – *Ips sexdentatus*, *Ips typographus* and *Dryocoetes autographus* (Ratz.).

MATERIAL AND METHODS

Insects were collected from three localities.

The first stand was located at an altitude of 1400 m above sea level in a forest on Zlatnite Mostove (part of the northern range of Vitosha Mountain, West Bulgaria). Adults of *Ips typographus* were found under the bark of dead spruce trees (*Picea abies* L.) in 2006.

Adults of *Ips sexdentatus* were collected from the bark of dead Scots pine trees (*Pinus sylvestris* L.) in 2008 from a second stand that was located at an altitude of 1230 m above sea level on Maleshevska Mountain (South-West Bulgaria).

Adults of *D. autographus* were found in 2008 under the bark of fallen and dead spruce trees in the forest reserve of Bistrishko Branishte (Vitosha Mountain). The stand was at 1550 m above sea level.

Dead pest adults were examined for fungal pathogens in 2008 and 2009 at the Department of Biological and Integrated Pest Control (Plant Protection Institute). Cadavers of insects were placed in a moist chamber to allow fungal reproductive structures to develop. Fungal pathogens were isolated into pure cultures on SDAY (Sabouraud dextrose agar with yeast extract) and were identified according to Samson et al. (1988) and Humber (1997) based on morphological characteristics both on the host and in the culture. Morphological characteristics of the isolates were studied by phenotypic methods. Colonies growing for 14 days at 23°C under daylight were checked on plates with PDA (potato dextrose agar) and SDAY. Conidia and conidiogenous cells were observed in smears and durable specimens with lactophenol and aniline blue using a transmission interference microscope BX60 DIC Olympus equipped with digital camera and Cell B image capture software with a calibrated Carl Zeiss micrometer.

RESULTS AND DISCUSSION

It was established that the examined mycoses on bark beetles were caused predominantly by the species *Beauveria bassiana* (Bals.-Criv.) Vuillemin. Two isolates (433 and 434 *B. bassiana*) from *Ips typographus*, three isolates (501, 502 and 503 *B. bassiana*) from *Ips sexdentatus* and one (559 *B. bassiana*) from *D. autographus* were obtained. One isolate each of the species *B. brongniartii* (Saccardo) Petch (marked 435) and *Isaria farinosa* (Holmsk.) Fries (marked 500) were isolated into pure cultures from *Ips typographus* and *Ips sexdentatus,* respectively. The three fungal species are anamorphs of Ascomycota belonging to Sordariomycetes: Hypocreales, Cordycipitaceae.

Dead insects placed in a moist chamber were covered after three days by dense mycelium of the fungus

Table 1. Size of conidia of *Beauveria bassiana* (Bals.-Criv.) Vuillemin isolates obtained from adults of bark beetles

Fungal isolate	Size of conidia (μm)		
	Length (minimum – maximum)	Width (minimum – maximum)	Average size (Length x Width)
433 *Beauveria bassiana*	1.04-1.91	1.13-1.56	1.34 x 1.32
434 *Beauveria bassiana*	0.95-1.91	0.95-1.65	1.28 x 1.25
501 *Beauveria bassiana*	0.95-1.56	0.78-1.56	0.28 x 1.27
502 *Beauveria bassiana*	0.86-1.72	0.43-1.72	1.16 x 1.02
503 *Beauveria bassiana*	1.30-1.99	0.69-1.65	1.63 x 1.11
559 *Beauveria bassiana*	1.56-2.17	1.30-1.82	1.87 x 1.60

Table 2. Colony growth of the isolates of *Beauveria bassiana* (Bals.-Criv.) Vuillemin, *Beauveria brongniartii* (Saccardo) Petch and *Isaria farinosa* (Holmsk.) Fries on PDA (potato dextrose agar) and SDAY (Sabouraud dextrose agar with yeast extract) during 14 days of cultivation at 23°C

Fungal isolate	Size of fungal colony on plates with PDA (mm)		Size of fungal colony on plates with SDAY (mm)	
	Diameter	Height	Diameter	Height
433 *Beauveria bassiana*	19.7	thin layer	21.3	3
434 *Beauveria bassiana*	17.7	thin layer	28.7	4
501 *Beauveria bassiana*	34.0	thin layer	20.3	2
502 *Beauveria bassiana*	27.3	thin layer	18.3	2
503 *Beauveria bassiana*	27.3	thin layer	20.7	2
559 *Beauveria bassiana*	21.7	thin layer	21.0	2
435 *Beauveria brongniartii*	20.7	thin layer	22.7	2
500 *Isaria farinosa*	34.0	thin layer	36.0	6

- white in the cases of *B. bassiana* and *B. brongniartii* and yellowish white in the case of *I. farinosa*. Mycelium started growing out through the inter-segmental membrane of the host.

Conidiogenous cells of *B. bassiana* were densely clustered in whorls, hyaline, smooth and short. In some smears and durable specimens of all fungal isolates, it was noticed that conidiogenous cells were branched, which made clusters thicker. Conidiogenous cells had globose (isolates 433, 434, 503 and 559 *B. bassiana*) or flask-like base (501, 502 *B. bassiana*) terminating in narrow extended denticulate apex. The growing apex repeatedly formed conidium and re-grew just below the new conidium, giving a distinctly zig-zag appearance (rachis) which in some cases reached 12.86 μm (isolate 501 *B. bassiana*) or even up to 18.44 μm (isolate 502 *B. bassiana*). Conidia were one-celled, hyaline, thin-walled, hydrophobic, globose in shape, very similar in size in different isolates – from 1.16 x 1.02 μm (isolate 502 *B. bassiana*) to 1.87 x 1.60 μm (isolate 559 *B. bassiana*) (Table 1). Conidiogenous cells of the isolates of *B. bassiana* have been observed in cultures often to be aggregated into clavate synnemata. The synnemata of the isolate 501 *B. bassiana* reached 10 mm in length in older cultures with SDAY.

Conidia of *B. bassiana* isolates (Table 1) were smaller in size than conidia of the species reported by Brady (1979a) and by Evlakhova (1974) as 2-3 x 2-2.5 μm and as 2-3 μm in diameter, respectively, but they were similar in size to conidia of the isolate 426 *B. bassiana* with dimensions 1.8-2.0 μm in diameter (Draganova et al., 2007).

On SDAY, *B. bassiana* isolates formed round raised colonies with powdery surface, with pigmentation from white to cream, reverse of the colonies with pale cream pigmentation. Only the isolate 501 *B. bassiana* released pink pigment into the media until the 10th day, but it paled and gradually disappeared and the redish reverse of the colonies became pink-tanned. On PDA the colonies were round, flat, like a hyaline film from radial growing mycelium, downy in the centre. The colonies of *B. bassiana* isolates grew slowly. After 14 days at 23°C and exposure to daylight they were between 17.7 and 34.0 mm in diameter on PDA and between 18.3 and 28.7 mm on SDAY (Table 2).

Isolate 435 *B. brongniartii* had morphological traits very similar to the morphology of *B. bassiana* isolates. Its colonies on PDA were round, flat, like a hyaline film from radial growing mycelium, downy in the centre. Isolate 435 *B. brongniartii* grew better on SDAY,

forming round raised colonies with powdery surface, with cream pigmentation, and cream-pigmented reverse of the colonies. The size of colonies reached 20.7 mm in diameter on PDA and 22.7 mm on SDAY after 14 days of cultivation at 23°C. Conidiogenous cells were loose, in small groups, with flask-shaped basal part and long denticulate rachis with conidia formed on the denticles. The conidia of isolate 435 were one-celled, hyaline, smooth, ellipsoidal, measuring 1.86 x 1.08 µm. These dimensions were rather smaller than those reported by Brady (1979b) – 2.5-4.5 x 1.5-3 µm.

Glare and Inwood (1998) had described morphological and genetic characterisation of *Beauveria* spp. from New Zealand. They classified the isolates with conidia longer than 3 µm as *B. brongniartii,* and isolates with shorter and spherical conidia as *B. bassiana*.

Using a gene-genealogical approach to investigate molecular phylogenetic diversity of *Beauveria* and several presumptively related *Cordyceps* species, Rehner and Buckley (2005) analyzed 86 isolates from diverse geographic origins, habitats and insect hosts. They reported *B. brongniartii* isolates (one of them originating from Belgium and with insect host belonging to Coleoptera, Rhizophagidae) with smaller dimensions of conidia (2.1-2.9 x 1.9-2.3 µm).

The isolate marked 500, obtained from dead adults of *Ips sexdentatus,* was identified as *Isaria farinosa*. Its colonies on PDA were not very different from colonies of *B. bassiana* and *B. brongniartii*. They were round, flat, like a hyaline film from radial growing mycelium, downy in the centre. In our opinion, PDA was not suitable for this species. On SDAY, the colonies of the isolate 500 *I. farinosa* were round, raised, with fluffy surface, yellowish to orange pigmentation, the reverse of the colonies of yellowish orange. The size of the colonies reached 34 mm in diameter on PDA and 36 mm on SDAY after 14 days of cultivation at 23°C. Conidiophores arise from dense basal felt mycelium of the colonies, making them seem granular. Conidiophores were septate, hyaline, bearing groups of swollen lateral cells and whorls of conidiogenous cells. Conidiogenous cells were flask shaped phialides, smooth-walled, with average dimensions 5.36 x 1.07 µm, with cylindrical base tapering into a distinctly thin neck. Conidia of the isolate were one-celled, hyaline, smooth, lemon-shaped, with average dimensions 1.88-2.14 x 0.79-1.36 µm, borne on the tip of the phialides, forming dry chains.

The morphological traits of the isolate 500 *I. farinosa* were similar to earlier descriptions of the species *I. farinosa* (major synonym *Paecilomyces farinosus*) (Evlakhova, 1974; Brady, 1979c; Humber, 1997).

In conclusion, although the present study was preliminary, the results contribute to our knowledge of the diversity of entomopathogenic fungi on bark beetles in Bulgaria.

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Entomopatogene gljive koje se u prirodi javljaju kod tri vrste potkornjaka (Coleoptera: Curculionidae) u Bugarskoj

REZIME

Potkornjaci (Coleoptera: Curculionidae: Scolytinae) pripadaju jednoj od najštetnijih grupa šumskih insekata, te je delovanje njihovih prirodnih neprijatelja, patogena, parazitoida, parazita i predatora od velikog značaja.

Biodiverzitet entomopatogenih gljiva koje se javljaju kod potkornjaka u Bugarskoj malo je istraživan. Cilj ovog početnog ispitivanja bio je da se pronađu, odrede i prouče morfološke karakteristike gljiva kao entomopatogena koji se javljaju u prirodnim populacijama tri vrste potkornjaka (Curculionidae) – *Ips sexdentatus* Boern, *Ips typographus* (L.) i *Dryocoetes autographus* (Ratz.).

Uginuli adulti su prikupljeni ispod kore stabala *Pinus sylvestris* i *Picea abies* poreklom sa planina Meleševska i Vitoša. Patogene gljive su izolovane kao čiste kulture na podlozi SDAY (Sabouraud dextrose agar with yeast extract) i određene na osnovu morfoloških osobina kako na domaćinu, tako i u kulturi. Morfološke osobine izolata proučavane su fenotipskim metodama.

Izolati gljiva dobijeni sa uginulih adulta *Ips sexdentatus, Ips typographus* i *D. autographus* pripadaju vrstama *Beauveria bassiana* (Bals.-Criv.) Vuillemin, *Beauveria brongniartii* (Saccardo) Petch i *Isaria farinosa* (Holmsk.) Fries (anamorf Ascomycota, Sordariomycetes: Hypocreales, Cordycipitaceae). Dat je opis morfoloških osobina izolata.

Ključne reči: Potkornjaci; *Ips sexdentatus; Ips typographus; Dryocoetes autographus;* entomopatogene gljive; *Beauveria bassiana; Beauveria brongniartii; Isaria farinosa;* morfološke osobine; pojava u prirodi