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BIODIVERSITY OF ARTHROPOD PATHOGENS IN THE BIAŁOWIEŻA FOREST

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Abstract: In the years 2002–2004 strains of *Bacillus thuringiensis* and 37 species of entomopathogenic fungi were isolated and identified in the Polish and Belarussian parts of Białowieża Forest (BF). Mitosporic fungi and bacteria dominated in litter superficial soil layer, forest, litter and floor vegetation whereas entomophtoralen fungi prevailed in bushy undergrowth layers and tree crowns. The dominant species *Beauveria bassiana* was observed in forest floor, subcortical habitats on dead trees, meadows and rushes. The species *Entomophthora israelensis*, *Beauveria* cf. *bassiana*, *Paecilomyces suffultus* and *P. tenuipes* were for the first time described as insect pathogens in BF. Entomophthorales seem to hold much greater part than mitosporic forms in the whole diversity of entomopathogenic fungi. Relatively rich sets of these fungi recognised in BF during last decades confirm the predestination of this area as highly significant refuge for other groups of arthropod pathogens, and it should encourage scientists to widen their research and contribute to a rather scarce knowledge in this field.

Key words: arthropod pathogens, biodiversity, *Bacillus thuringiensis*, entomopathogenic fungi, Białowieża Forest

INTRODUCTION

The Białowieża Forest (BF) is a large area of pristine woodland which despite multigenerational human interference has retained its natural character and its numerous parts still represent primeval lowland forest. It makes the largest and best preserved complex of deciduous and mixed forests in the European Temperate Zone,

which covers in total 1,500 km^2 (625 km^2 in Poland; 875 km^2 in Belarus). To preserve the unique nature the entire area of BF various regimes of protection have been implemented. In the Polish part the Białowieża National Park (Białowieski Park Narodowy – BPN) occupies 10502 ha while the remaining part constitutes the Forest Promotive Complex (FPC) of a special protective status with numerous partial and strict reserves and zones of limited human interference (Okołów 1999; Szujecki 2002). The Belorussian section of 30,000 ha was turned into the State National Park "Belavezhskaya Pushcha".

There were numerous factors that have enabled development of rich natural associations of biota in this vast region with small scattered settlements, difficult for penetration by man. Great differentiation of forest and swamp plant communities caused by mosaic of moraine depositions and peculiar water systems make favorable habitats for rich diversity of animals, fungi and microorganisms, this being accompanied by various biogeographical elements related to mutual influences of subarctic, Atlantic and continental climates characteristic for this area. Apart from hunters and wild bee's keepers these unique values have also attracted naturalists and scientists with unflagging interest for more than 200 years. Results of their investigations have been listed in consecutive volumes of "Białowieża Bibliography" (Bibliografia białowieska), edited successively by the Białowieża National Park.

In 2003 a new teamwork project on biodiversity of arthropod pathogens occurring in both parts of BF was established between the Institute of Plant Protection in Poznań and the Institute of Plant Protection Minsk Region in Priluki. In 2004 S. Bałazy from the Research Center for Agricultural and Forest Environment, Polish Academy of Sciences in Poznań also joined the program. The aim of this paper is to present initial results of these investigations.

MATERIALS AND METHODS

The investigations reported here were carried out with similar methods in both parts of BF. The main objects of the surveys were poliophagous insects, subcortical entomofauna of dying trees with particular emphasis on bark beetles infesting Norway spruce, and other insects occurring on the forest floor vegetation and undergrowth. The Belorussian team studied mortality of epigeic insects being caused by fungi on plots of 0.5 × 0.5 m, while the Polish group used research plots of size 2 × 2 m. Litter and layers of mineral soil were checked throughout in accordance with rules applied in forestry in routine of autumn search for primary forest pests. Dead insects collected on research plots were subjected to detailed examination and isolation of entomopathogenic microorganisms. The Belorussian researchers isolated pathogenic bacteria and fungi from soil samples using the bait insect method (Zimmermann 1986). Apart from litter and soil the Polish group examined leaves and stems of undergrowth plants up to 2.5 m, the height up to which many insects died from fungous and other infections. In the Polish part of BF non-forest ecosystems such as rushes, wetlands and meadows, and some cultivated fields of woodless Polana Białowieska were also considered in the research. Bacteria were isolated from dead insects by inoculation of media in Petri dishes using highly diluted suspensions of the internal content from dead hosts. Fungi were isolated by direct sowing of spores taken from dead insects or mites on me-

dia or by point inoculation of media with small fragments of non-sporulating aerial mycelium developing on internal tissues of the host. The following media were used: Sabouraud dextrose agar (SDA), potato dextrose agar (PDA), Sabouraud dextrose enriched with egg yolk agar (SDEYA) and combinations of these media with rice, yeast extract or in some cases with addition of dead insect fragments. Representative cultures of pathogens were stored in the culture collections maintained in both Institutes.

RESULTS

In 2003, in the Belorussian part of the BF, investigations were conducted in nine forest sections. In total 73 samples were analyzed and 27 isolates of entomopathogenic microorganisms were obtained. Bacteria constituted 52% while fungi 48% of isolates (Tab. 1). Most microorganisms were isolated from samples collected in spruce forests and the least from maple and pine forests. The fungus *Beauveria bassiana* was isolated from more than half of soil samples while *Paecilomyces fumosoroseus* (Wize) Brown et Smith and *Metarhizium anisopliae* (Metsch.) Sorok. appeared only in single samples. *Bacillus thuringiensis* Berliner was the only bacterial pathogen isolated from dead insects. This species occurred most frequently in soil samples collected in spruce stands whereas in samples from pine and mixed deciduous forests dominated by maple it was found only occasionally. On dead bark beetles under bark of Norway spruce the most frequent fungus was *B. bassiana,* whereas *P. fumosoroseus* and *M. anisopliae,* were observed in few cases.

In the Polish part of BF (Tab. 2) the hyphomycetes *Paecilomyces farinosus* and *B. bassiana* dominated in material from litter and *P. tenuipes* frequently accompanied them. In one research section of black alder forests few insects were infected with *P. suffultus,* one was mummified by the mycelium of *Sesquicllium candelabrum* and a few individual spiders were covered with coremial mycelia of *Gibellula leiopus* and *G. pulchra*. Evaluation of the occurrence of fungal pathogens on arthropods in litter of research plots revealed during autumn of the years 2002–2004 from 17 to 37 cases of mycoses. *P. farinosus* and *B. bassiana* dominated in all samples and reached up to 63% and 21%, respectively. *P. tenuipes* was subdominant and constituted 10% while all other species were only accessory together amounting to about 7%. Different proportions of particular fungal pathogens were recorded in meadow and rush ecosystems, where in autumn

Table 1. Entomopathogenic microorganisms occurring in the Białowieża Forest (Belarus part) in 2003

No.	Section No.	No. of samples	No. of isolated entomopathogens		
			total	fungi	bacteria
1	714 maple forest	11	1	–	1
2	741 mixed forest	15	3	2	1
3	773 spruce forest	7	9	2	7
5	801 spruce forest	19	6	1	5
6	803 pine forest	11	1	1	–
7	715 mixed forest	3	2	2	–
8	688 mixed forest	4	2	2	–
9	587 spruce forest	3	3	3	–

Table 2. Species of entomopathogenic fungi found in the Białowieża Forest area in 2002–2004

Fungal species	Host arthropods, habitats and other comments	Research plots	
		research plot	outside res. plot
Chytridiomycetes			
Chytridiopsis typographi Weiser	*Ips amitimes*, *I. duplicatus*, *I. typographus* – adult beetles in their galleries		x
Entomophthorales			
Batkoa apiculata (Thaxter) Humber	*Diptera*, *Hemiptera* and *Lepidoptera*, rarely insects of other orders – adults of different species; mostly in forest floor vegetation and undergrowth	x	x
Conidiobolus coronatus (Costantin) Batko	*Dipt.*, *Sciaridae* – preimaginal and adult individuals, mites *Dendrolaelaps spp.*; in bark beetle galleries on spruce, reared in laboratory		x
Conidiobolus sp.	*Tipulidae* and *Limoniidae* – adults; in forest floor vegetation in moist sites	x	x
Entomophaga tenthredinis (Fres.) Batko	*Tenthredinidae* (larva of *Dolerus* sp.) in mid forest meadows – rare		x
E. tipulae (Fres.) Humber	*Tipulidae* and *Limoniidae* – adults; in forest floor in moist sites.	x	x
Entomophthora muscae (Cohn) Fres.	*Anthomyidae* – on adults, sometimes epizootic, seldom singly on other calyptrate flies	x	x
Ent. israelensis Ben-Ze'ev	*Itonididae* – in bushy undergrowth of forests, on single host specimens		x
Ent. planchoniana Cornu	Aphids – mostly on forest undergrowth and in mid-forest meadows	x	x
Erynia ovispora (Nowakowski) Nowakowski ex Remaud. et Henneb.	*Calliphoridae*, *Scatophagidae* and possibly other unidentified calyptrate flies, in swampy forests and rushes	x	x
Erynia variabilis (Thaxter) Remaud. et Henneb.	Small adults of *Nematocerous* flies, most frequently *Psychodidae*, in streams and digs, just over water surface	x	x
Eryniopsis caroliniana (Thaxter) Humber	*Tipula* and *Nephrotoma* species – on single adult individuals in moist sites		x
Furia americana (Thaxter) Humber	*Calliphoridae* adults in arboreous undergrowth, only one specimen		x
Neozygites fresenii (Nowakowski) Batko	As above; from July to September		x
Pandora dipterigena (Thaxter) Humber	Common on *Nematocera* in forest floor and meadows	x	x
P. echinospora (Thaxter) Humber	*Lauxaniidae* and *Dryomyzidae* adults; only on single specimens in forest floor; late summer and autumn		x
P.myrmecophaga (Turian et Wuest) Humber	*Formica polyctena*, on ant-workers at a group of nests in BPN, only single specimens, although occurring continuously		x
P. lipai Bałazy, Eilenberg et Papierok	*Rhagonycha lignosa* – adults in bushy undergrowth of mixed forests, in June	x	x
P. muscivora (Schroeter) Bałazy	Common on different adult flies from end of May till autumn except prolonged dry weather periods	x	x

Fungal species	Host arthropods, habitats and other comments	Research plots	
		research plot	outside res. plot
P. neoaphidis (Remaud. et Henneb.) Humber	Aphid species, common in farmland and field margins; rare and only in single host specimens in forests; from June to autumn.	x	x
Zoophthora aprophorae (Rostrup)	Mirid bugs (*Dicyphus* or *Stenodema* sp.) on *Stachys silvatica* in one site in BPN.		x
Z. autumnalis Bałazy	*Dryomyzidae* – adults; till September till first days of November.	x	x
Z. crassitunicata Keller	*Malthodes* spp. – adults; turn of May – June; rare in deciduous forest undergrowth.		x
Z. ichneumonis Bałazy	*Ichneumonidae* – adults; on leaves of bushy undergrowth; from end of May till September; locally frequent, even during dry summer periods.	x	x
Z. nematoceris Bałazy	On adults of small nematocera; in forest floor and undergrowth, in spring (May-June) often frequent, in summer and autumn singly.	x	x
Z. psyllae Bałazy	*Trioza urtica*e not frequent, in turn of July.		x
Z. radicans (Brefeld) Batko	*Operophthera brumata* – caterpillars, May-first days of June; in deciduous forests.		x
Hyphomycetes			
Beauveria bassiana (Bals.) Vuill.	Insects, seldom on spiders; everywhere common.	x	x
B. cf. bassiana	*Auchenorrhyncha* – selected small species; moist grasslands; in late summer and autumn, often epizootic.	x	
Gibellula leiopus (Vuill. in Maublanc) Mains	Spiders (juvenile and adults); in meadows, seldom in forest undergrowth.	x	x
G. pulchra (Cavara) Saccardo	Spiders (as above); in moist meadows and rushes, common from summer till late autumn.	x	
Hirsutella sp. (*brownorum* or *nodulosa*)	*Dendrolaelaps sp.* (Acari), single specimens in bark beetle galleries on Norway spruce.		x
Lecanicillium spp. and allied forms (formerly *Verticilium* sect. *Prostrata*)	On different insect and mites, sometimes on spiders in subcortical feeding sites and in litter, on leaves of plants – the species to be identified.	x	x
Paecilomyces farinosus Brown et Smith	Different insects, mostly *Lepidoptera*, *Hymenoptera* and *Diptera*, seldom single spiders; in forest litter abundant, in meadows on single specimens; in fall often epizootic.	x	x
P. suffultus (Petch) Samson	*Bibio sp.* – larvae; in the litter of flood-plain forest (Circaeo – Alnetum); Narewka River valley.	x	x
P. tenuipes (Peck) Samson	Caterpillars and pupae of *Lepidoptera*; deciduous forest litter; common in late summer and autumn.	x	x
Sesquicillium candelabrum (Bonorden) W. Gams	Larvae of predaceous beetles in deciduous forest litter; not numerous in autumn.	x	x

species of *Gibellula* genus infecting spiders and *B. bassiana* usually dominated. Subdominant group consisted of *Verticillium* species with features characteristic for the section *Prostrata* (Gams 1971) (presently regarded as a new genus *Lecanicillium*), but this did not regularly occur on each of research plots. *P. farinosus* and *P. tenuipes* were recorded occasionally. Moreover, after dry summer of 2003 in rushes, spider mycoses caused by *Gibellula* spp. were rare even in autumn. However, in the same area relatively great number of small plant hoppers (on average 8 individuals/1 m^2) infected by an unidentified strain of *Beauveria* related to *B. bassiana* was recorded.

In galleries of cambiophagous insects under the bark of dead spruces *B. bassiana* occurred regularly but in insignificant numbers as compared to the overall density of bark beetles and predacious and parasitoid arthropods present in this habitat (less than 1% of mortality cases). Single host individuals were sometimes infected by *P. farinosus* and *Lecanicillium* spp. After 2–3 weeks of rearing experimental material in closed cages or cylinders some representatives of individuals insect infected with *B. bassiana* augmented quickly up to 3–5%, and numerous mites mostly of the group of Gamasida died from infections by *Hirsutella* spp. From among a relatively great number of these obtained fungous strains *H. nodulosa* Petch was so far identified. In some cases the entomophthoralean species *Conidiobolus coronatus* (Costantin) Batko appeared on numerous larvae, pupae and adults of sciarid flies and on mites *Dendrolaelaps* spp., seldom also on single larvae of beetles.

The first mycoses caused by entomophthoralean fungi were recorded at the end of May and beginning of June. *Entomophaga tipulae, Erynia ovispora, Zoophthora nematoceris* occurred on *Diptera* adults and *Z. radicans* on *Operophthera brumata* caterpillars. The more frequent late spring appearance of most entomophthoralean species was observed from June 10 till the end of this month. At this time of the vegetative season the species *Pandora muscivora* on numerous brachycerous flies occurred, *Zoophthora lipai* on *Rhagonycha lignosa* adults, and out of forest areas also *Entomophthora muscae* on *Anthomyidae* and *Pandora neoaphidis* on some aphid species culminated. Appearance of entomophthoralean species during summer was dependent upon the precipitation rate in the course of time. One week of dry and sunny weather clearly decreased the frequency of entomophthoralean fungi and such decline lasted usually till 3rd to 5th day after heavy rains. During such periods of drought only *Zoophthora ichneumonis* was guite regularly recorded on adult ichnemonids. Single individuals of typically autumnal species *Z. autumnalis* appearing on dipterans (mostly from the *Dryomyzidae* family) were collected from September until late fall, and even at the beginning of November. The remaining species were found occasionally at different times. In the present research epizootic caused by these pathogens were not recorded.

Several species have been recorded for the first time in BF, such as *Entomophthora israelensis,* seen occasionally on adult itonidid gnats, *Beauveria cf. bassiana* on *Auchenorrhyncha* collected in wetlands, *Paecilomyces suffultus* on bibionid larvae and *P. tenuipes* on butterfly pupae. Further new species for the BF region are expected to be found among unidentified yet isolates of hyphomycetes of the genus *Hirsutella* and prostrate forms of *Verticillium* (*Lecanicillium*).

Moreover, several *Geotrupes silvaticus* beetles collected in tree stands in both the Polish and the Belorussian part of BF were studied. The results showed that 17% of examined

beetles were infected with *Entomopoxvirus* which was previously discovered in the Polish part of BF in the population of *G. silvaticus* (Lipa and Bartkowski 1971).

DISCUSSION

When compared with the thoroughly studied problems of geobotany and well recognized communities of macro- and mezofauna the knowledge on arthropod pathogens of BF seems rather scarce. In early 1930s Karpiński (1935) in his studies on natural mortality of bark beetles infesting Norway spruce considered some fungi, protozoans and nematodes as agents responsible for infective or invasive diseases of *Ips typographus* and allied species. Siemaszko (1937) has identified the fungi as *Beauveria bassiana,* whereas protozoans and nematodes remained unidentified. In a similar study continued since 1960s by Bałazy (Bałazy 1966; 1968; Bałazy et al. 1967) apart from numerous species of parasitoids and predators of the above bark beetles, also were identified: a protozoan *Gregarina typographi* Fuchs, nematodes *Contortylenchus diplogaster, Polymorphotylenchus typographi* and *Parasitorhabolitis obtusa* and fungi *Chytridiopsis typographi* Weiser (formerly regarded as protozoans under the generic name *Haplosporidium*), *B. bassiana, P. farinosus, V. lecanii* (Zimm.) Viégas (recently transferred to a new genus *Lecanicillium* (Zare and Gams 2001) and *M. anisopliae.* The hyphomycete species *B. bassiana* and *"Cephalosporium thripidum"* Petch (which actually represents the genus *Lecanicillium*) have also been reported by Okołów (1970) as pathogens of the bark beetle *Hylesinus crenatus* Fabricius. Batko (1964) in his studies on entomophthorales, described a new fungal species *Zoophthora phalloides* found on the aphid *Microlophium carnosum* on nettle in BPN. On calyptrate flies Rozsypal (1966) found also a newly described species *Pandora vomitoriae* (Rozsypal 1966) (originally under a generic name *Zoophthora*). Extensive study on this group of fungi continued by Bałazy (1993) allowed enlarging the number of species known from BF to 52. In arthropods Lipa (1967) discovered several new species of protozoans, mainly of the group *Gregarinomorpha,* and described a new microsporidian species *Plistophora geotrupina* Lipa which parasitizes *Geotrupes stercorarius* L. (Lipa 1968).

Based on the available bibliography it can be stated that the only groups of arthropod pathogens recognized are terrestrial forms of entomopathogenic fungi. Up to now 77 of their species, e.i. 35% of the total list for the whole Poland were known from all ecosystems of BF and 61% of their number were reported from the Polish forests (Bałazy and Cysewski 2003; Bałazy 2004).

Preservation of unrestrained development and persistence of biological species and their associations in their natural habitats is the major aim of various forms of territorial protection. The most abundant populations can be found in natural environments of multispecies populations in either protected areas or regions of less intensive human activity. In this respect BF belongs to the one of European regions with the greatest number of surveys of living organisms in natural environment. Therefore, arthropod fauna is well described and it makes easier to conduct investigations on pathogens related to that group of invertebrates. So far this opportunity availed mostly mycological research (Majewski 1994; Bałazy 1994; 2004) whereas other groups of pathogens (viral, bacterial, nematic and protozoan) represent a great potential for the development of investigations. Information on arthropod pathogens and their biologi-

cal control is significant for currently developing integrated pest management (IPM) programs, because of possibilities of the use of infective agents in pest control.

CONCLUSIONS

1. In the years 2002–2004, strains of *Bacillus thuringiensis* and 37 species of entomopathogenic fungi were isolated and identified in the Białowieża Forest. Mitosporic fungi and bacteria dominated in litter, superficial soil layer, forest, litter and floor vegetation whereas entomophthoralean fungi prevailed in bushy undergrowth layers and tree crowns.
2. The most ubiquitous is *Beauveria bassiana* which occurs as a dominant (or co-dominant) species in forest floor, subcortical habitats on dead trees, meadows and rushes.
3. *Paecilomyces farinosus* was most numerous in forest litter, two *Gibellula* species co-dominated with *B. bassiana* in grasslands and rush habitats, whereas *Hirsutella* species developed on mites in bark beetle's galleries.
4. Entomophthorales seem to hold much greater part than mitosporic forms in the whole diversity of entomopathogenic fungi. Relatively rich sets of these fungi recognized in BF during last decades confirm the predestination of this area as highly significant refuge of other groups of arthropod's pathogens. This should encourage scientists to widen their research, and contribute to a rather scarce knowledge in this field.

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POLISH SUMMARY

BIOLOGICZNA RÓŻNORODNOŚĆ PATOGENÓW STAWONOGÓW W PUSZCZY BIAŁOWIESKIEJ

W latach 2002–2004 na terytorium białoruskiej i polskiej części Puszczy Białowieskiej prowadzono badania nad patogenami stawonogów. Po stronie białoruskiej z 73 prób wyizolowano 27 izolatów mikroorganizmów, wśród których dominowały bakterie. W części polskiej badania skupiły się tylko na patogenach grzybowych. Izolowano 37 gatunków grzybów. Dominantami w ściółce leśnej i powierzchniowej warstwie gleby były grzyby mitosporowe, natomiast w podszycie i koronach drzew – grzyby owadomorkowe. W zbiorowiskach łąkowych i szuwarowych dominowały gatunki grzybów z rodzaju *Gibellula*. Gatunki *Entomophthora israelensis*, *Beauveria* cf. *bassiana*, *Paecilomyces suffultus* i *P. tenuipes* nie były dotychczas opisane i izolowane w Puszczy Białowieskiej.