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INSECT PATHOGENS WITH SPECIAL REFERENCE TO PATHOGENS OF BARK BEETLES (COL., Scolytidae: *Ips typographus* L.). PRELIMINARY RESULTS OF ISOLATION OF ENTOMOPATHOGENIC FUNGI FROM TWO SPRUCE BARK BEETLES IN SLOVENIA

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Abstract:
This paper deals with the most important groups of insect pathogens, i.e. viruses, bacteria, fungi, nematodes and protozoans (microsporidia). We describe their basic characteristics, virulence, method of infecting or attack on the host, signs of illnesses in an affected host, pathogen survival in the outside environment, and the use of bioticcontrol of economically damaging insects. Particular importance is placed on bark beetle pathogens, which have been found in natural populations of hosts, particularly in the large spruce bark beetle (*Scolytidae: Ips typographus* L.). We also present our experience in studying entomopathogenic fungi on the species *Dryocoetes autographus* and *Orthotomicus laricis* in Slovenia.

Key words: insect pathogens, beetles pathogens, entomopathogenic fungi, *Scolytidae, Ips typographus*, biocontrol agents

PATOGENI ŽUŽELK S POUDARKOM NA PATOGENIH PODLUBNIKIH (COL., Scolytidae*: Ips typographus* L.). PRELIMINARNI REZULTATI O IZOLACIJI ENTOMOPATOGENIH GLIV IZ DVEH SMREKOVIH PODLUBNIKOV V SLOVENIJI

Izvleček:
Prispevek obravnava najpomembnejše skupine patogenov žuželk, to so virusi, bakterije, glive, gliste ter praživali (mikrosporidiji). Prikazuje njihove osnovne značilnosti, virulenco, načine okužbe ali napada gostitelja, znamenja obolenja okuženega gostitelja, preživetje v zunanjem okolju ter njihovo uporabo za biotično zatiranje gospodarsko škodljivih žuželk. Posebno pozornost posveča patogenom podlubnikov, ki so bili ugotovljeni v naravnih populacijah gostiteljev, posebej v velikem smrekovem lubadarju (*Scolytidae: Ips typographus* L.). Podaja nekaj izkušenj v raziskavi entomopatogenih gliv na vrstah *Dryocoetes autographus* ter *Orthotomicus laricis* v Sloveniji.

Ključne besede: patogeni žuželk, patogeni hroščev, entomopatogene glive, *Scolytidae, Ips typographus*, biotični pripravki

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VSEBINA

CONTENTS

1 INTRODUCTION
UVOD

Among pathogens affecting insects or entomopathogens, there are a number of organisms that cause disease and death in their hosts. The exceptional importance of this insufficiently researched area is expressed in two ways. Firstly, pathogens are often the decisive factor in the regulation of natural populations of insects. Therefore, without an understanding of specific insect pathogens and the forms of their parasitic relationship with the host it is impossible to recognize and understand the dynamics of insect populations. Secondly, an understanding of pathogenic organisms which parasitize insects gives us opportunity to manipulate populations of insects in various ways, either by identifying pathogens to use as classical agents of biotic control (biotic suppression) or by increasing the natural populations of existing pathogenic insects in the environment and using them as biopesticides.

During the period of global warming the question arises as to how changes in various ecological factors will influence insects as pathogen hosts and as pathogens themselves, and how this will affect the interactive relationship between pathogens and hosts. Researchers determined more than half a century ago that abiotic and biotic stress increases the sensitivity of insects and predisposes them to associated infections or to attack by a variety of pathogens simultaneously. The synergistic or antagonistic activity of various pathogens is the result of these infections (STEINHAUS 1958, LYSENKO 1959, op. cit. WEGENSTEINER *et al.* 1996).

We have begun research on entomopathogens of phytophagic insects of the Coleoptera group and present the preliminary results of our research here.

2 GROUPS OF INSECT PATHOGENS
SKUPINE PATOGENOV ŽUŽELK

The most important groups of entomopathogens are viruses, bacteria, fungi, nematodes, and protozoans (microsporidia). The techniques for studying these organisms have advanced greatly over the past decade - as evidenced by the fact that contemporary taxonomy of the above organisms is based on comparative sequencing of ribosomal RNA, which allows all living organisms to be divided into three superkingdoms: Bacteria, Archaea, and Eukarya (MADIGAN *et al.* 2003). Viruses without a protein envelope (»non-occluded viruses«) are studied using an electron microscope, while viruses with a protein envelope

(»occluded viruses«) and bacteria are studied using a light microscope with an oil-immersion objective. Bacteria and fungi are grown on a culture medium under sterile conditions. Fungi, protozoans (microsporidias), and smaller species of nematodes are visualized using a light microscope, while larger nematodes can be seen with the naked eye. We will present the basic characteristics and most important taxons of the above-mentioned groups.

2.1 VIRUSES
VIRUSI

Viruses are non-cellular organisms with a genome of nucleic acids, which are only capable of replication within the cells of a host using the metabolic system and ribosomes of that host. There they form so-called virions (a virus in infective form, consisting of an RNA particle within a protein covering) which preserves their genome and allows the transmission of the virus to other cells. Because viruses neither breathe nor are sensitive to outside stimulus, and that they neither move nor grow, early taxonomists did not classify them as living organisms. However, viruses do reproduce and are capable of adapting to new hosts. Newer taxonomic studies have therefore classified viruses among living organisms and have changed the definition of a living organism: »An organism is a fundamental element with a continuous individual evolutionary history« (http://www:meb.uct.ac.za/tutorial/virwhat.html).

The most important taxonomic indices for the classification of viruses are their hosts, particle morphology, and type of genome. The taxonomic system includes orders (with the ending −*virales*), families (−*viridae*), sub-families (−*virinae*), genera (−*virus*), and species. The first classification, which is based on the hosts in which the viruses reside, divides viruses into groups which reside in prokaryotes (Bacteria, Archaea) and eukaryotes (Eukarya − algae, plants, fungi, animals) (according to the International Committee on the Taxonomy of Viruses 1966, MURPHY *et al.* 1995). It is now customary to name species of viruses by their English name after their host, basic symptom, and genus (groups) of the virus (LOEBENSTEIN, 1995, op cit. AGRIOS 1995).

The following families are found among invertebrates (and insects): Baculoviridae (genera *Nucleopolyhedrovirus* and *Granulovirus*), Birnaviridae (genus *Entomobirnavirus*, species Drosophila X virus), Iridoviridae, Parvoviridae (genus *Iteravirus*, species *Bombyx mori densovirus*), Poxviridae (genus *Entomopoxvirus A*, species *Melolontha melolontha entomopoxvirus*), Reoviridae (genus *Cypovirus*, species *Bombyx mori cypovirus* 1) and others (MURPHY *et al.* 1995). Viruses of insects usually enter the host through the mouth opening. The protein envelope of the virus then degrades in the digestive tract of the insect and releases virus particles (virions). The virions invade and multiply in the cells of the wall of the digestive organs of the host. Massive replication of the virus continues in the fat body, hemocytes, and the hypodermis of the insect. The death of the host occurs within three to ten days. After death, the body of the host degrades and releases millions of virions (virus particles). Viruses that have a protein coat ("occlusion body") can survive for years under suitable conditions. Entomopathogenic viruses can be grown *in vitro* only on living insects or in tissue culture. Preliminary isolation of the virus is carried out by culturing the live infected host in distilled water for a few days, centrifuging it, and obtaining a partially clean sample, which should contain the virus, which is then used to innoculate a healthy host. This is followed by re-isolation and identification using a phase-contrast microscope or serological techniques (http://www.lubilosa.org/Engl01a.pdf, MURPHY *et al.* 1995).

Symptoms: viruses mainly infect the larval stage of insects, which then become lighter in color, limp, and weak. Larvae become darker after death. The symptoms caused by individual groups of viruses can be specific (e.g. following infection with baculovirus the inside of the body of the host liquefies, a whitish discharge may be excreted, larvae may hang by their prolegs, infected larvae are smaller than uninfected and so on) (*ibid.*).

The most important entomopathogenic families of viruses are Baculoviridae and Poxviridae.

BACULOVIRIDAE

Representatives of this family were the first insect viruses to be used as classical bioinsecticides. These are still the most widely used insect viruses in biotic controls. They are divided into two genera *Nucleopolyhedrovirus* (NPV) and *Granulovirus* (GV).

They have double stranded DNA and are protected by a protein coat, which makes them quite durable. The majority of these viruses infect insect larvae which feed on contaminated food. The invasion of a virulent baculovirus into the digestive tract (midgut), hematolymphatic system, and fatty tissues causes paralysis of the host followed in 3-10 days by the death of the insect.

a) The *Nucleopolyhedrovirus* (NPV) genus includes around 280 species of virus, which are from 0.5 to 1.5 μm in size and are shaped in the form of a rounded cube or a hexagonal polyhedron. Infection by these species occurs through the fatty component of the hypodermis, trachea, and digestive tract. The hosts of this genus include around

120 species of butterfly (Lepidoptera) and Hymenoptera, especially wood wasps. Viruses of this group are highly specialized to a particular host. These viruses are very hardy, in fact, the crystal protein structure of their protein envelope coat can allow them to survive for many years outside of the host, provided that they are not exposed to the sun. Biocontrol agents based on NPV and sold for commercial purposes are: *Autographa californica* NPV; *Lymantria dispar* NPV; *Malacosoma disstria* NPV; *Mamestra brassicae* NPV; *Neodiprion sertifer* NPV; *Spodoptera* NPV and *Heliothis* NPV.

Various products on the basis of specific NPV (LpNPV) are used as possible bioinsecticides for the control and suppression of the gypsy moth (*Lymantria dispar* L.), such as the well-known Gypchek in the USA, Disparvirus in Canada, Biola in the Czech Republic, and Virin−Ensh in Russia (GLARE *et al.* 1998).

b) The genus Granulovirus (GV) includes around 65 species which are oval or ovoid in shape. The hosts are usually caterpillars. As they have mechanisms similar to those of the previous group, they are also very hardy outside of the host. Biocontrol agents using this genus are: *Cydia pomonella* GV (Codling moth virus) and *Phthorimaea operculella* GV (potato tuberworm virus).

c) Some taxosonomies define yet another group called the Group C Baculoviruses. These have double-stranded DNA, are from 22 to 30 ηm in size, and are not hardy. The route of infection and effects on the insect are similar to those of the previous group. The viruses of this group are limited to arthropods, generally to larvae and adult insects of the Coleoptera and Hymenoptera groups, as well as to mites. *Baculovirus oryctes* is used in the biocontrol of beetles of the *Oryctes* genus. *B. oryctes* is released from the infected live host in the form of virions. These come into contact with other adults during copulation or through contaminated food eaten by the larva or adult. This species of virus is not hardy.

POXVIRIDAE

The most important genus of the Poxviridae family is the *Entomopoxvirus A*. Species of this genus are from 5 to 20 μm in size and have special »inclusion bodies«, which are important in identification. Infection occurs through contaminated food. After infection of the insect the virus invades the fatty tissue. The death of the insect is much slower that with baculovirus infections. Hosts are caterpillars (Lepidoptera), flies (Diptera), beetles (Coleoptera), as well as crickets, grasshoppers (Orthoptera). This is a poorly studied group http://www.lubilosa.org/Engl01a.pdf. Viruses represent the most numerous group of microorganisms and are becoming an increasingly important tool in microbial genetics and applied genetic engineering, thus further increasing the possibilities of their use as bioinsecticides (MADIGAN *et al.* 2003).

2.2 BACTERIA
BAKTERIJE

Theses are classified as Prokaryotes, superkingdom Bacteria, and include more than 40 phyla. The classification of bacteria is based on the shape of the bacteria, its ability to produce spores, its method of obtaining energy (glycolysis for anaerobic and cellular respiration for aerobic), the need for nutrition, Gram reactions, and sequencing of the genome (16S ribosomal RNA) (http://microbe.org/microbes/bacterium1.asp). The most important groups of bacteria are Proteobacteria, Firmicutes, Actinomycetes, Mycobacteria, Corynebacteria, Spirochetes, Mycoplasma, Chlamydiae, Cyanobacteria, Mitochondria, and Chloroplasts.

The most important entomopathogenic bacteria are found in the Firmicutes phylum, Bacilli class, Bacillaceae family, and genus *Bacillus*. There are also other species of bacteria in insects, but the majority of these only cause infections in already weakened or injured insects, or in insects infected with other pathogens.

Entomopathogenic bacteria infect insects by the introduction of infected food into the digestive tract. From the digestive tract the bacteria spread to the hemocoel where they multiply again. Hosts of entomopathogenic bacteria are insect groups like butterflies (Lepidoptera), beetles (Coleoptera), and flies (Diptera). These can be grown *in vitro* relatively easily on a specific hard or liquid medium. Preliminary isolation of bacteria is carried out so that the outside of the host is sterilized by dipping it in 90 % ethanol for a few seconds, then placing it into a 50 % sodium hypochlorite solution for 3–4 min., after which the insect is rinsed in sterile water, the insect is dissected using sterile techniques, the body parts are placed on an appropriate culture medium and are cultured for 24 hours at 30 ^{o}C. This is followed by identification of the bacteria.

Symptoms: following infection with bacteria the larvae remain a normal color, after death they darken to a brown-black, they are often limp, and they never liquefy. The most important biocontrol agents are from the *Bacillus* genus.

BACILLUS SPP.

Bacillus species are aerobic, Gram-positive, one-celled, usually bacilliform (rod-shaped) bacteria which produce spores. Infection of insects occurs through ingestion of bacteria or their spores. The majority affect the larval stage of phytophagic insects. The bacteria of this genus enter the hemocoel, reproduce there, and generally cause lethal septicemia. A toxin penetrates into the epithelial cells of the digestive tract and causes paralysis of the muscles of the mouth and digestive apparatus of the insect. Feeding stops, the insect vomits its food or has diarrhea. The host dies within 30 min. to 24 hours. Death may be caused by starvation of the insect. The susceptibility of the host to the infection is dependent on the pH reactions of the digestive tract of the host - which bacteria of the *Bacillus* genus specialize in.

The most important entomopathogenic bacteria is *Bacillus thuringiensis* Berliner, named by the birth province of Berliner - the Thuringia in Germany. The toxicity of *B. thuringiensis* to flour moths was determined in 1911 in Berlin in Germany. Research showed that during sporulation *B. thuringiensis* produces long protein crystals representing a proto-toxin, which is transformed into a toxic polypeptide (delta-endotoxin) in the alkaline environment (above 9.5 pH) of the host digestive tract (http://helios.bto.ed.ac.uk/bto/microbes/bt.htm).

B. thuringiensis (Bt) has been used as a commercial insecticide in France since 1939, and since the 1950's in the USA and New Zealand (GLARE *et al.* 1998). Due to the relative ease in obtaining bacteria of the *Bacillus* genus in large quantities through fermentation processes, a number of biocontrol agents on the basis of *Bacillus thuringiensis*, *B. sphaericus* and *B. popilliae* have been developed. These are sold as preparations with a variety of trade names, such as: Bt, Biobit®, Dipel®, Javelin®, Dipel, Foray, and Vectobac™12AS. (GLARE *et al.* 1998, http://helios.bto.ed.ac.uk/bto/microbes/bt.htm). Commercial Bt is a powder, which contains a mixture of dry spores of *B. thuringiensis* and toxin in the form of crystals. Commercial products on the basis of *B. thuringiensis* (Bt) represent around 1 % of the total »agrochemical« market (which also includes fungicides, herbicides and insecticides) http://helios.bto.ed.ac.uk/bto/microbes/bt.htm. Specific sub-types (varieties or sub-species) have been developed for various hosts: *Bacillus thuringiensis* var. *kurstaki* mostly infects butterflies (Lepidoptera), *B. thuringiensis* var. *israelensis* infects mosquitoes and other types of Diptera, and *B. thuringiensis* var. *tenebrionis* infects beetles (Coleoptera). It is characteristic for the *Bacillus popilliae* species not to produce toxins in the infection cycle. Japanese beetle larvae (*Popillia japonica* Newm.) consume spores of this bacteria, which then multiply and within 3-5 days fill the digestive tract of the larvae. The spores develop in the walls of the digestive tract and sporulate further in the hemolymph. Within 14-21 days after infection, the body of the larvae is swollen and a white-cream color (milky disease). Following the death of the host, the spores are released into the soil and establish a lasting contamination of the soil (http://www.lubilosa.org/Engl01a.pdf).

2.3 FUNGI
GLIVE

Fungi belong to the superkingdom Eukaryota. Fungi which live in or on insects are usually pathogens called entomogenic fungi. Entomogenic fungi, in terms of life forms, range from commensals to mutualists to ectoparasites, ranging from those which do not seriously afflict their arthropod hosts to those which are lethal pathogens to their host. Therefore, various phrases are used for entomogenic fungi, such as entomopathogens, entomogenic mutualistic symbionts, entomogenic ectoparasites, and entomogenic endoparasites (EVANS 1988).

Entomogenic fungi, which are found in all of the larger groups of the Fungi kingdom, are believed to include around 750 species (EVANS / LATGE, 1988, KIRK *et al.* 2001). Fungi of the Trichomycetes and Laboulbeniales groups, which are often isolated from insects, are not explicitly insect pathogens (WEIR/ HAMMOND 1997, THAXTER 1971, http://lsb380.plbio.lsu.edu/Netscape.comp.laboul.page).

Fungi of the Chytridiomycota, Oomycota and Zygomycota phyla are important insect pathogens. An important species from the Oomycota group is *Lagenidium giganteum*, which mostly parasitizes mosquito larvae and is the basis of the Laginex™ AS Biotical Larvicide. Entomophthoraceae (genera *Entomophthora*, *Erynia*, *Massospora*, *Neozygites*, *Zoophthora*) is an important family from the Zygomycota groups. The species *Entomophthora maimaiga* has been successfully employed as a biocontrol agent against caterpillars (*Lymantria dispar* L.) in America and New Zealand due to its high pathogenicity (HAJEK *et al.* 2000a, HAJEK *et al.* 2000b, GLARE *et al.* 1998). Fungi from the Ascomycota phylum (genera *Ascosphaera*, *Cordyceps*, *Torrubiella*, *Hypocrella* and its anamorph *Aschersonia* sp.) often cause epizootics. In the Hyphomycetes group (phylum Deuteromycota) there are a number of important entomopathogenic fungi of the genera *Beauveria*, *Culicinomyces*, *Hirsutella*, *Metarhizium*, *Nomuraea*, *Paecilomyces* and *Verticillium* (KIRK *et al.* 2001, DOMSCH *et al.* 1993).

Entomogenic fungi can have a complicated development cycle with both a sexual (teleomorph) and asexual (anamorph) form, such as fungi from the Entomophthoraceae group (e.g. *Entomophaga grylli*), or a simple development without a sexual form, such as fungi of the Hyphomycetes group (e.g. genera *Metarhizium* and *Beauveria*). Spores of the majority of entomogenic fungi propagate on the outer surface of the host body, on the cuticle. Some burrow into the cuticle of the insects through chitinolysis or through mechanical pressure. Some fungi, such as *Metarhizium anisopliae* (Metschn.) Sorok., need a moist environment to develop and therefore infection of the hosts proceeds orally (DOMSCH *et al.* 1993). The fungi quickly multiply in the hemocoel, either through hyphal division or through the formation of asexual and sexual spores. Sexual and asexual spores spread through the body of the host. The insect dies due to suffocation or to physical blockage of circulation in the host (due to intensive colonization of the host by mycelia from the fungi) or due to poisoning by entomotoxins released by the fungi. The cadaver quickly dries out, because the fungi continue to grow using the available moisture and food provided by the host body. The spores are then actively or passively passed to new host insects. Fungi from the Entomophthoraceae group survive unsuitable conditions as sexual spores.

Growing entomogenic fungi *in vitro* is difficult: some groups need a complex media to develop, while other groups cannot be grown under laboratory conditions. Some produce mycelia, but these do not sporulate (HUMBER 1998).

Symptoms of infection are the following: fungi may infect all stages of the insect, after death the cadaver is dried out, never liquefied. In insects with a soft body the body is covered with mycelia, while for insects with a hard exocuticle mycelia grows between individual hard segments and fixes itself to the substrate. Spores can form on the surface of the body of infected insects. In a very dry environment there may be no external symptoms of infection.

ENTOMOPHTHORACEAE

There are two main species from the Entomophthoraceae family used to produce mycoinsecticides: *Entomophaga praxibulli* and *Zoophthora radicans*. The first species was introduced in the USA in order to control grasshoppers, the second species is used as a classical biocontrol agent to suppress aphids in Australia (http://www.lubilosa.org/Engl01a.pdf).

HYPHOMYCETES

Hyphomycetes are comprised of fungi, which have no known sexual stage, and multiply by conidia. Newer studies have confirmed that despite a lack of sexual stage, fungi from this group do exchange genetic material. These processes are called parasexual exchange of genetic material. The majority of species of this group can be simply and easily grown on culture medium *in vitro* (DOMSCH *et al.* 1993).

Biocontrol agents are: *Metarhizium*, *Beauveria* and *Verticillium lecanii*. The most frequently used fungus from the first genus is *Metarhizium anisopliae* (grown on a rice substrate). A widely used biocontrol agent in China, Europe and America, is a preparation using two fungi, *Beauveria bassiana* (Bals.) Vuill. and *B. brongniartii* (Sacc.) Petch (DOMSCH *et al.* 1993). *Verticillium lecanii* (Zimm.) Viégas is one of the most important entomogenic hyphomycetes and was isolated from scale insects and aphids, thrips, species of the Diptera, Hymenoptera, and Lepidoptera orders, and mites. A biopesticide against aphids has been developed from the fungus *V. lecanii*; it is used in greenhouses in Europe (*ibid.*).

In Slovenia, the fungi content in dead insects found in caves has also been studied (ZALAR *et al.* 1997, GUNDE–CIMERMAN *et al.* 1998).

2.4 NEMATODES
GLISTE

Nematodes belong to the kingdom Animalia, to the group of Aschelminthes (unsegmented worms) and to the phylum Nematoda (= Nemata). This group includes the largest number of individuals, since it includes 80 % of all multicellular animals (UREK / HRŽIČ 1998). Nematodes are unsegmented worm-like animals with a tough outer cuticle. They are found in all moist environments abundant in organic matter (they are saprophytes, plant or animal parasites and predators). Entomopathogenic nematodes are usually larger than other entomophathogenic organisms and some species can even be seen with the naked eye. Nematodes are classified as entomopathogens above all because some species of nematode are important vectors of entomopathogenic bacteria (THOMAS / POINAR 1979, AKHURST 1980). The first records of the pathogenic activity of nematodes versus insects are from the 17th century, while the possibilities of using nematodes to control insects had already been reported in 1930 (SMART 1995). In 1929, two researchers Glaser and Fox discovered a nematode on a golf course in New Jersey that attacked the Japanese beetle (*Popillia japonica*) (GLASER / FOX 1930, op cit. SMART 1995). Steiner described the nematode as the species *Neoaplectana* (= *Steinernema*) *glaseri*. Today we know that this nematode has a symbiotic relationship with the bacteria *Bacillus popilliae*, which is a pathogen to the host. Glaser grew this nematode in large quantities in the laboratory, applied it to 73 locations in nature and confirmed that it had pathogenic activity against the Japanese beetle (*Popillia japonica*). The nematodes remained in the natural environment for around 9 years following their introduction into the soil (GLASER / FARRELL 1935, GLASER *et al.* 1940, op cit. SMART 1995).

There are believed to be 8 orders of Nematodes with a parasitic relationship to insects. Species from 9 families of nematode (Allantonematidae, Diplogasteridae, Heterorhabditidae, Mermithidae, Neotylenchidae, Rhabditidae, Sphaerulariidae, Steinernematidae and Tetradonematidae) are known to attack insects causing death, sterility, or changes in the development of the host (http://nematode.unl.edu/wormepns.htm;http://nematode.unl.edu/epn/epnintro.htm).

Symptoms of attack: the majority of nematodes can be seen through the cuticle of the host. Among groups of Rhabditidae nematodes the body of the insect changes color after death from cream-colored to grey (for Steinernema groups) or reddish (for Heterorhabditis groups).

Nematodes can be successfully grown *in vitro* in live insects or on artificial media with added macerated host insects (http://www.lubilosa.org/Engl01a.pdf).

Preliminary isolation of nematodes: individual nematodes, most of which can be seen with the naked eye, are transferred from the cadaver and placed in a sterile suspension, then into a drop of sterile water, which is then heated and formalin added as a fixative. The nematode is then transferred to a drop of lactophenol or glycerol, then onto a glass slide and viewed through a microscope. The sample can then be identified.

Mermithidae represent a large group of obligatory parasites. They burrow through the cuticle in the second larval stage of insects and develop there until the fourth larval stage, by which time they have filled the body cavity of the host, as evidenced by a bloated abdomen. The larvae then burrow out of the host through the cuticle and continue their development in the soil or in water. Biocontrol agents against mosquitoes and grasshoppers have been developed from this group of nematodes (http://www.lubilosa.org/Engl01a.pdf).

Species from the families Heterorhabditidae and Steinernematidae are known as rabditid nematodes. Chemotropism allows rabditid nematodes in the third larval stage to attract appropriate insects. Nematode larvae enter the insect through the mouth opening or through the anus, from which they burrow through the gut wall of the host into the hemocoel. Some species can enter the host directly through the cuticle. The nematode releases a very virulent bacteria (*Xenorhabdus* spp.) into the hemocoel of the host. The host dies from septicemia in 2 to 10 days. After 10 days the larvae leave the cadaver and spread into the outside environment. They need water in order to spread.

Entomopathogenic nematodes (EPNs) (Heterorhabditidae and Steinernematidae) are potential bioinsecticides for butterflies (particularly the species *Thaumatopoea pityocampa*) (http://nematode.unl.edu/epn/epnino.htm; TARASCO / TRIGGANI 2002).

2.5 PROTOZOA (MICROSPORIDIA)
PROTOZOA (MICROSPORIDIA)

Protozoans cover a variety of one-called organisms with an as yet undefined taxonomic classification and are divided into various phyla. According to some authors, protozoans are in the kingdom Protista, sub-kingdom Protozoa, which is divided into the Sarcomastigophora, Ciliophora and Apicomplexa phyla. (http://www.cbs.umn.edu/class/spring2000/biol/2012/protozoa.htm). Some taxonomists believe that species of the phyla Microspora belong in Microsporidia (Protozoa), which is divided into two classes (Dihaplophasea and Haplophasea). The basic criteria used in newer taxonomy is the absence or presence capsules in the spore packet, the number of spores in the packet and the nuclear cycle, which can be uninuclear or dikariont throughout the whole development cycle or may alternate between uninuclear to diplokariont (SPRAGUE *et al.* 1992).

Protozoans have a number of hosts, the most numerous of which are arthropods and mammals, but they are especially prevalent as obligatory pathogens in insects. The genera of beetles that are frequently effected are: Nosema, Chytridiopsis, Pleistophora, Chytridiopsis and Unikaryon (SPRAGUE *et al.* 1992).

The death of the host takes place very slowly. Protozoans are not very pathogenic, but they do slow the development and fertility of the host. Chronic infections are more common than lethal ones. Protozoans multiply asexually within the digestive tract or fat body of the host via multiple or binary fission. The fusion of two gametes can form a zygote which divides repeatedly. Hosts carry protozoan spores in their digestive tract which have polar capsules that can develop into tubes that can penetrate gut cell walls. The host can die due to very high rates of multiplication of the protozoan. After death the host releases spores that are highly resistant. Transmission of protozoan spores is also possible through the host's eggs.

Symptoms of attack: the host larvae are underdeveloped and weak. They become stiff and slowly decline. The death of the insect usually occurs due to other causes and the attack of the protozoan only hastens death. Protozoans can only be grown *in vitro* on live insects. Preliminary isolation of protozoans is carried out such that the fat body, the Malpighian tubes, gut epithelia, and hemolymph are examined with a phase-contrast or light microscope. Mature protozoan spores are easily seen, although they can be confused with fungal sporulations. The protozoan can then be identified. The following are used as biocontrol agents: *Microspora*, *Nosema*, *Vairimorpha* and *Malamoeba*. The species *Nosema locustae* is used to control grasshoppers and the species *Vairimorpha necatrix* to suppress a number of butterflies (Lepidoptera) (http://www.lubilosa.org/Engl01a.pdf).

3 PATHOGENS IN BARK BEETLES
PATOGENI V PODLUBNIKIH

The bark beetles are the most harmful biotic factor found in European forests with a high percentage of conifers on non-arable land (VITÉ 1989, EIDMANN 1992). There are relatively few species of primary bark beetles that inhabit and afflict healthy trees. In Europe, the species primarily seen are bark beetles on Norway spruce (*Picea abies* (L.) Karst.), such as the large spruce bark beetle (*Ips typographus* L.) and in the past few years the eight-toothed spruce bark beetle (*Ips amitinus* Eichhoff), and bark beetles on silver fir (*Abies alba* Mill.), such as *Pityokteines curvidens* (Germar), *P. spinidens* (Reitter) and *Cryphalus piceae* (Ratzeburg). The most economically damaging bark beetle is *Ips typographus*, which usually inhabits weakened Norway spruce throughout Europe (LINDELÖW / SCHROEDER 2001; NETHERER *et al.* 2003, JURC 2003, STERGULC / FACCOLI 2003). Despite the damage bark beetles cause, little is known about their pathogens (ESCHERICH 1923, POSTNER 1974, WEISER 1961, PURRINI / FÜHRER 1979, MILLS, 1983, STEPHEN *et al.* 1993). Pathogens have only been studied in a few hosts. Many authors have reported on pathogens of spruce bark beetles like *I. typographus* (FUCHS 1915, op cit. WEGENSTEINER *et. al*. 1996, BALAZY 1966, WEGENSTEINER / WEISER 1995). Pathogens of the six-toothed spruce bark beetle, *Pityogenes chalcographus* (L.), were studied by Purrini and Führer (1979). Purrini (1980) also dealt with pathogens of the species *Dryocoetes autographus* (Ratzeburg) and *Hylurgops palliatus* (Gyllenhal). Pathogens of the small spruce bark beetle, *Polygraphus poligraphus*, have been isolated from the natural host population in the past few years (WEISER / HÄNDEL / WEGENSTEINER / ZIZKA 2002). Pathogens of the fir bark beetle (*Pitykteines spinidens*) have also been studied (WEISER *et al.* 1995). Field studies of the influence of pathogens on the development of biotical methods of controlling bark beetles (e.g. lesser pine-shoot beetle − *Blastophagus minor* Hartig) in Spain have shown good results from the application of suspensions of pathogenic fungi *Beauveria bassiana* and *Verticil-*

lium lecanii, as well as the pathogenic nematode *Heterorhabditis megidis*, on trap trees. The insecticide phenitrothion was used as the control (RUIZ–PORTERO *et al.* 2002).

In the first studies of pathogens, findings of individual pathogens in their hosts was reported on, but the biology, ecology, and epidemiology of these pathogens were not studied until the mid 1990's. Similarly, there was not a lot of attention given to the pathogen-host relationship and the influence of the pathogen on the reproduction of the host. Studies of pathogens of bark beetles and their influence on the natural population of bark beetles are becoming ever more important, particularly due to the possibility of using these pathogens in biotical methods of regulating populations of economically harmful species of bark beetle.

Microsporidia (Microspora) are the most studied group of bark beetle pathogens. Representatives of this group cause a slow decline in natural populations of bark beetles (WEISER *et al.* 1995, 1998). In 1979, two researchers, Purrini and Führer, reported on parasites of the Amoebidae (*Malamoeba scolyti* Purrini) and Ophryocystidae (*Menzbieria chalcographi* Weiser) groups on the six-toothed spruce bark beetle *Pityogenes chalcographus* (PURRINI / FÜHRER 1979). One year later they described *Malamoeba scolyti* in the bark beetles *Dryocoetes autographus* and *Hylurgops palliatus* (PURRINI 1980). The most important protozoan pathogens of bark beetles are found in the genera *Nosema*, *Pleistophora*, *Chytridiopsis* and *Unikaryon* (WEISER *et al.* 2002). Species of the genus *Chytridiopsis* are typical and frequent pathogens of a number of bark beetles, where they develop in the epithelium of the midgut and form characteristic cysts with thick walls. Other representatives of microsporidia (e.g. species of the family Unikaryonidae) are usually specialized for particular species of bark beetles. For example, the species *Unikaryon minutum* parasitizes *Dendroctonus frontalis* (KNELL / ALLEN 1978), *U. montanum* and *I. typographus* (WEISER *et al.* 1998). A related species is *Canningia spinidentis*, which parasitizes the fir bark beetle (*Pitykteines spinidens*). The parasite *C. spinidentis* lives in the midgut of the bark beetle and later moves into the muscles, gonads, and fat body of the host (WEISER *et al.* 1995). A new species of parasite (*Unikaryon polygraphi*) was found in Austria in the adult small spruce bark beetle (*Polygraphus poligraphus*) in a number of columnar cells of the midgut, longitudinal and spherical muscles, and in the Malpighian tubes (WEISER *et al.* 2002). Studies of all developmental stages of bark beetles have confirmed that protozoan parasites usually appear in the adult phase of the host and that there is horizontal transmission of parasites of this group through excrement which the adult bark beetle excretes into the egg gallery before it moves to a new host tree (WEGENSTEINER / WEISER 1996).

A few studies have also reported on the importance of fungi as pathogens of bark beetles, particularly Hyphomycetes fungi (BALAZY 1966, MOORE 1971). BALAZY (1966) emphasizes the entomopathogenic importance of the species *Beauveria brongniartii*. MOORE (1971) cites data on the lethal effect of fungi of the genus *Beauveria* and other species of bacteria on bark beetles of the genus *Dendroctonus.* The primacy of certain spruce bark beetles is connected to their association with blue–stain fungi, mostly of the genera *Ceratocystis* and *Ophiostoma*. However, the interspecific relationship between fungi, bark beetles, and host trees is still not completely clear (CHRISTIANSEN *et al.*, 1987, CHRISTIANSEN / BAKKE 1997, RAFFA 1991, op cit. KROKENE / SOLHEIM 1996).

In Slovenia, pathogens of bark beetles have not been studied, although they have been mentioned in publications which give a general overview of the most common species of bark beetle in Slovenia and population control methods of using these species (TITOVŠEK 1988). This author cites the literature data on entomopathogenic bacteria, entomopathogenic fungi, sporozoans, and microsporidia. *Aerobacter scolyti*, *Escherichia klebsiellae–formis* and *Servatia marescens* bacteria are found in the larvae and pupae of the large elm bark beetle *Scolytus scolytus* (Fabricius) and the small European elm bark beetle *Scolytus multistratius* (Marsham). The pathogenic fungus *Paecilomyces varioti* is found in *Dryocoetes autographus* (Ratzeburg) and *Leperesinus varius* (Fabricius) species. Bark beetles are also hosts for widespread fungal species such as *Beauveria bassiana* and *Spicaria farinosa*. Various pathogens (Sporozoa, Protozoa) are found in the digestive tract of bark beetles, namely *Haplosporidium typographi*; microsporidia *Plistiphora scolyti* – infect *S. scolytus*; *Nosema typographi* sporadically appear in *I. typographus*; *Nosema curvidens* is frequently seen in *Pityokteines curvidens*. Gregarine (vermiform protozoans parasitic in insects and other invertebrates: Gregarinida, order Gregarinida, sporozoan) species *Gregarina typographi* and *Schizogregarina menziliera chalcographi* have been found on *Pityogenes chalcographus* (TITOVŠEK 1988). Due to newer taxonomy, particularly for pathogens of the Microsporidia group, the use of some Latin names for groups and species used by the cited author has now been abandoned.

4 PATHOGENS IN *IPS TYPOGRAPHUS*
PATOGENI V *IPS TYPOGRAPHUS*

The first studies of pathogens from a natural population of *Ips typographus* and the connection between the appearance of the pathogen and ecological factors were made in Austria and the Czech Republic. They found that the level of infection with protozoa in *I. typographus* in the natural population was relatively low. There was also a large variability in the density of the population of pathogens dependent on the time and location of bark beetle collection and the developmental stage of the host (WEGENSTEINER *et al.* 1996, WEGENSTEINER / WEISER 1996). *I. typographus* is the host to several groups of pathogens. Entomopoxvirus was found to be present in a sample of epithelia from the midgut of *I. typographus*. This was followed by protozoans of the Microsporidia group: the species *Chytridiopsis typographi* Weiser was found in the cells of the midgut, while the species *Nosema typographi* Weiser was found in the fat body, connective tissue and the gonads. The protozoan *Gregarina typographi* Fuchs, classified as Apicomplexa (Eugregarinida, family Gregarinidae), was isolated from the lumen of the midgut. The species *Malamoeba scolyti* Purrini (Amoebida) was found in parts of the midgut and excretory canals (WEGENSTEINER / WEISER 1996, WEGENSTEINER *et al.* 1996).

In studies of the *I. typographus* population from Austria and the Czech Republic from 1992-1994, all 15 locations showed frequent infections of the host with pathogenic fungi of the genus *Beauveria* (WEGENSTEINER *et al.* 1996). In the past few years, field studies and laboratory experiments have been conducted on the influence of pathogenic fungi on the development and reproduction of *I. typographus*. In Germany, field studies and later laboratory experiments using a combination of pheromone attractants and the pathogenic fungus *Beauveria bassiana* in a special traps showed a high level of virulence by the pathogenic fungus *B. bassiana* against *I. typographus*, as well as a high level of mycosis (91 %) and mortality in adult bark beetles (KREUTZ *et al.* 2000a). They also tested the transfer of *B. bassiana* spores, through which adult *I. typographus* are infected in pheromone traps, in a population in nature. They made a preparation on the basis of *B. bassiana*, which was named Boverol (KREUTZ *et al.* 2001). A similar field and laboratory study was carried out in Poland in 1998 using *B. bassiana* to suppress *I. typographus* (KREUTZ *et al.* 2000b).

5 ENTOMOPATHOGENIC FUNGI FROM TWO SPECIES OF SPRUCE BARK BEETLES (*DRYOCOETES AUTOGRAPHUS* AND *ORTHOTOMICUS LARICIS*) IN SLOVENIA
ENTOMOPATOGENE GLIVE DVEH VRST SMREKOVIH PODLUBNIKOV (*DRYOCOETES AUTOGRAPHUS IN ORTHOTOMICUS LARICIS*) V SLOVENIJI

In 2003, a study was carried out on a stand of Norway spruce in the Bled Forest Management Unit in order to determine the importance of removing the bark from tree stumps to protect the forest from dangerous bark beetles. We aimed to analyze the presence of entomopathogenic populations of fungi in the most frequently encountered species of bark beetles found under the bark of tree stumps without stripped bark and under the bark of the root back of stripped tree stumps.

5.1 METHODS
METODE

5.1.1 Sampling materials
Vzorčenje materiala

From 16 July to 4 November 2003 (16. 07, 26. 09., 25. 10, 04. 11.) we collected entomofauna from 60−80 year old stands of Norway spruce, at three locations, from under the bark of tree stumps and from root backs of tree stumps of Norway spruce (*Picea abies* (L.) Karst.). Samples were collected from tree stumps where the bark was stripped off after felling (materials were collected from under the bark of the root back) and from tree stumps where the bark was only partially removed. The first location was Pod gradom (near Bled Castle), about 500 m above sea level, and the second location was at Čerteša (near Bohinjska Bela), about 700 m above sea level, and the third location was Meja dolina (Pokljuka) (forest association *Rhytidiadelpho lorei – Piceetum* M. WRABER 1953 n. nud.), at about 1300 m above sea level. At each location we collected entomofauna from six tree stumps (from three partially bark stripped stumps and from three completely stripped stumps), from four samplings we sampled a total of 24 stumps. The objective of the study was to determine the importance of bark stripping from tree stumps in protecting the forest from dangerous bark beetles. We particularly wanted to analyze the presence of entomopathogenic fungi in common species of bark beetles.

5.1.2 Isolation of fungi
Izolacija gliv

We have developed a method of isolating entomopathogenic fungi from freshly dead and dried adult bark beetles.

The procedure is the following: if the recently dead bark beetle showed no visible symptoms of fungal infection, we cultured the host for a few days on a moist medium. We noted possible sporulation of fungi, any spores and hyphae were transferred onto a slide in a carrier (lactophenol) to which we added specific colors and identified the fungi. In cases where we saw sporulation of fungi on the host we used sterile techniques to transfer them into various media (2 % malt agar, PDA agar, Sabouraud agar) with antibiotics, incubated them at 20–25 °C, and examined all cultures with a stereoscopic microscope. In cases where the host had died a long time before we carried out the procedure using a surface sterilization of the host by dipping them in 70–90 % ethanol for a few seconds, then in 50 % sodium hypochlorite for 3–4 min., the bark beetles were then rinsed in sterile water, dissected using sterile techniques, the body parts were transferred to a suitable growth medium and were cultured for possible growth and sporulation of fungi. We analyzed a total of 1216 adult bark beetles, including two of the most common species. Identification of fungi followed. Defining the species was based on effective determination keys (THAXTER 1971, EVANS / LATGE 1988 DOMSCH *et al.* 1993, HUMBER 1998).

5.2 PRELIMINARY RESULTS
PREDHODNI REZULTATI

Determination of entomofauna and processing of results is currently under way. Taxonomic work has shown that two species of bark beetle *Dryocoetes autographus* (Ratzeburg) and *Orthotomicus laricis* (Fabricius) are predominant under the bark.

We determined that the content of isolated fungi differs depending on whether the fungus is isolated from recently dead individuals or long dead individuals. The numbers of individual species of fungi and their species composition was also dependent on the time of sampling of the bark beetles. The predominant fungi found were Hyphomycetes (Pictures 1, 2).

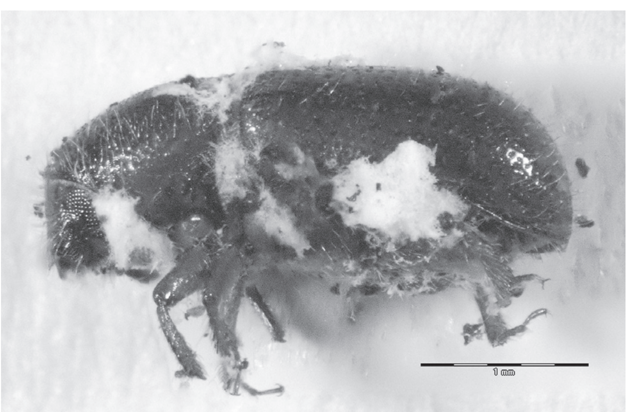

Figure 1. Dried bark beetle Dryocoetes autographus with an outgrowth of fungal mycelia (picture M. Jurc)
Slika 1. Posušen podlubnik Dryocoetes autographus iz katerega izrašča podgobje glive (foto. M. Jurc)

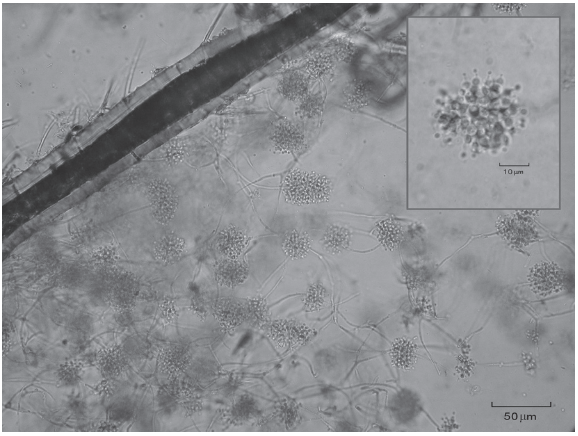

Figure 2. One of the isolated fungi, *Beauveria bassiana* (Bals.), from the Hyphomycetes group (picture D. Jurc)
Slika 2. Ena od izoliranih gliv, *Beauveria bassiana* (Bals.), iz skupine Hyphomycetes (foto. D. Jurc)

6 DISCUSSION AND CONCLUSION
RAZPRAVA IN ZAKLJUČEK

In the past ten years, particularly in the past few years, there have been a number of statements in the professional literature regarding direct and indirect evidence of global warming of the Earth. Meteorological measurements using world-wide standards clearly show that our planet has again begun to warm up (IPCC 1996).

A warmer climate, particularly warmer winters, will favor the appearance and development of certain diseases and pests such as beetles and aphids (STRAW 1995), as well as pathogenic fungi (BRASIER 1996). The appearance of new species of pests and pathogens that did not live in such areas previously, penetration of warm-weather species into new areals and their occupation of niches held by endemic species (and the dying out of endemic species), the spread of disease in animals and humans due to the increase in suitable vectors have all been forecast in response to global warming trends (SUTHERST 1995).

All of this means that in the future we will have to face known species, such as insects, which will have a different biology than those we have today, or new and unknown species that may cause grave economic damage to the environment.

On the other hand, throughout Europe and in other parts of the world there is an effort underway to preserve the productiveness and biodiversity of terrestrial ecosystems, particularly forests. Regulations have increased the amount of suitable habitats for primary saproxiles, represented mainly by bark beetles (SPEIGHT 1989, LINDELÖWN / SCHROEDER 2001, HEDGREN 2002). Where there are frequent windthrows and snowthrows and dry and hot summers, there will likely be a higher density of bark beetle populations and greater damage to forests. In the past 10 years we have been a close witness to the increase in damage-related and sanitary-related tree fellings in coniferous forests due to the »small, black menace«− bark beetles.

A greater overall effort to preserve biodiversity and create a suitable environment for the human population has resulted in legal regulations regarding bans on the use of chemical means for controlling or reducing economically damaging species of insects and other organisms. Therefore it is not by chance that the number of studies on parasites and pathogens of insects and the putting of these studies into practice has grown in the past 10 years.

I. typographus is still the most important economically damaging species in European forests and also one of the most interesting research topics (VITÉ 1989, EIDMANN 1992). The results of the studies on pathogens of the species *I. typographus* in Austria and the Czech Republic were unexpected: whether the studies on pathogens were carried out in protected areas or in managed forests the spectrum of pathogens found was very similar (WEGENSTEINER *et al.* 1996). They also found that the presence of pathogens of *I. typographus* was dependent on high density populations of bark beetles over a longer time period. The combined high frequency of pathogens of *I. typographus* (Entomopoxvirus − EPV, *Chytridiopsis typographus* and *Gregarina typographi*) was linked to intense abiotic stress in the study area (*ibid*.).

An important conclusion of the study on bark beetles was that the majority of bark beetle pathogens apparently spread through horizontal transmission. Thus, contact between the host and the pathogen and the introduction of the pathogen into the digestive tract of the bark beetles is important for the successful spread of pathogens within the bark beetle population. This problem appears not only in adults but also in the younger generation of bark beetles due to their specific methods of feeding. Due to their feeding in a relatively »sterile« phloem, younger individuals spend relatively short time in contact with the galleries of adult beetles, with infected adults, and with excrement that may contain a number of pathogens in various stages of development.

Due to the migration tendencies of species in the bark beetle family and the associated transmission of pathogenic organisms into new areals, it is necessary to stop simply monitoring the situation and to introduce studies of bark beetle pathogens over the entire areal of Scolytidae family in Europe. In Slovenia, studies are aimed at entomogenic species of fungi in important species of bark beetle.

It is necessary to acquire an overall understanding of the probable changes in the ecology of economically significant species of bark beetle, the epidemiology of bark beetle pathogens, and their interactions. The mentioned relationships could be clarified through experiments involving artificial infection of bark beetles by various pathogens.

Continual problems with bark beetles in managed forests and the unsolved problem of how to control them emphasize the importance of conducting research work in the field, while simultaneously offering a good opportunity to use methods of biotical control. The perspective offered by an understanding of the pathology of bark beetles is in the introduction of criteria to evaluate the threat to local populations of bark beetles with respect to the influence of pathogens. Therefore, the possible transmission of pathogens into populations of bark beetles should be studied. The great importance of bark beetles, particularly in conifer forests, emphasizes the importance of future studies of pathogens of this family.

Through an understanding of pathogens, as one of the most important natural regulators of natural populations of insects, we will better know and understand the population dynamics of insects. On the other hand, through an understanding of the pathogenic organisms that parasitize insects, a number of possibilities for manipulating populations of insects present themselves, e.g. by introducing pathogens as classical agents of biotical warfare or by increasing the natural population of pre-existing insect pathogens in the environment and using them as biopesticides.

Research efforts oriented towards the use of natural enemies and natural pathogens in controlling populations of targeted economically damaging species of insects are important in actively directing forest development in a dynamic and unpredictably changing environment.

7 POVZETEK

Najpomembnejše skupine entomopatogenov so virusi, bakterije, glive, gliste (nematode) ter praživali (mikrosporidiji). Prikazujemo najpomembnejše taksone omenjenih petih skupin entomopatogenov. Pri virusih so to družine Baculoviridae (rodovi *Nucleopolyhedrovirus* in *Granulovirus*), Birnaviridae (rod *Entomobirnavirus*, vrsta Drosophila X virus), Iridoviridae, Parvoviridae (rod *Iteravirus*, vrsta *Bombyx mori densovirus*), Poxviridae (rod *Entomopoxvirus A*, vrsta *Melolontha melolontha entomopoxvirus*), Reoviridae (rod *Cypovirus*, vrsta *Bombyx mori cypovirus* 1); pri bakterijah obravnavamo najpomembnejši rod *Bacillus* (z vrstami *Bacillus thuringiensis*, *B. sphaericus* in *B. popilliae*); pri glivah so pomembni predstavniki debel Chytridiomycota, Oomycota ter Zygomycota - poudarek je na skupinah Entomophthoraceae (vrsti *Entomophaga praxibulli* in *Zoophthora radicans*) ter Hyphomycetes (z vrstami *Metarhizium anisopliae, Beauveria bassiana* ter *Verticillium lecanii*); od glist so v parazitskem odnosu z žuželkami vrste iz devetih družin (Allantonematidae, Diplogasteridae, Heterorhabditidae, Mermithidae, Neotylenchidae, Rhabditidae, Sphaerulariidae, Steinernematidae in Tetradonematidae); od praživali so najpomembnejše iz rodov *Nosema*, *Chytridiopsis*, *Pleistophora*, *Chytridiopsis* in *Unikaryon*. Nadaljujemo s prikazovanjem osnovnih značilnosti posameznih pomembnih patogenov žuželk, njihove virulence, načinov okužb ali napada gostitelja, znamenj obolenj okuženega gostitelja, preživetja v zunanjem okolju ter njihove uporabe v sklopu biotičnega varstva pred gospodarsko pomembnimi škodljivimi žuželkami.

Glede na to, da predstavljajo podlubniki v evropskih gozdovih z veliko zastopanostjo iglavcev na neustreznih rastiščih najpomembnejšo skupino škodljivih biotskih dejavnikov, se temeljiteje lotevamo pregleda raziskovalnih dosežkov patogenov podlubnikov, ki so bili ugotovljeni v naravnih populacijah gostiteljev. Ugotavljamo, da je sorazmerno malo znanega o patogenih podlubnikov. Najpogosteje se na podlubnikih pojavljajo patogene glive (*Beauveria bassiana* in *Verticillium lecanii)*, gliste (*Heterorhabditis* sp.) ter mikrosporidiji (z rodovi *Nosema*, *Pleistophora*, *Chytridiopsis* in *Unikaryon*). Posebej obravnavamo patogene velikega smrekovega lubadarja (*Ips typographus* L.). To vrsto parazitirajo predvsem virusi (skupina Entomopoksvirusov), sledi skupina Microsporidia (vrste *Chytridiopsis typographi*, *Nosema typographi* in dr.) ter patogene glive rodu *Beauveria*.

Podajamo nekaj začetnih izkušenj v raziskavi entomopatogenih gliv na vrstah *Dryocoetes autographus* ter *Orthotomicus laricis* v Sloveniji. Opisujemo način vzorčenja entomofavne pod skorjo panjev 60-80 let starih smrek na treh lokacijah v GGO Bled v letu 2003. Determinacija entomofavne in obdelava rezultatov je v teku. Taksonomsko delo je pokazalo, da pod skorjo panjev prevladujeta dve vrsti podlubnikov, *Dryocoetes autographus* (Ratzeburg) ter *Orthotomicus laricis* (Fabricius). Ugotavljamo, da je sestava izoliranih gliv v primeru izolacije gliv iz odmrlih osebkov drugačna od sestave gliv, izoliranih iz že dlje časa odmrlih osebkov. Številčnost posameznih vrst gliv in njihova vrstna sestava je bila odvisna tudi od časa vzorčenja podlubnikov. Prevladujejo glive iz skupine Hyphomycetes (predvsem vrsta *Beauveria bassiana*).

Izredna pomembnost entomopatogenov se izraža v dveh aspektih: po eni strani so patogeni pogosto odločilni dejavnik v regulaciji naravnih populacij žuželk; po drugi strani pa poznavanje patogenih organizmov, ki parazitirajo žuželke, omogoča številne manipulacije s populacijami žuželk bodisi z vnašanjem patogenov kot klasičnih agensov biotičnega zatiranja bodisi s povečanjem naravnih populacij že prisotnih patogenov žuželk v okolju ter njihovem izrabljanju kot biopesticidov.

Slovenska inačica teksta je na voljo v Gozdarski knjižnici, Večna pot 2 v Ljubljani kot sestavni del študijskega gradiva za predmet Varstvo gozdov, ki se izvaja na Oddelku za gozdarstvo in obnovljive gozdne vire Biotehniške fakultete Univerze v Ljubljani (JURC 2004).

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