

PATHOGENS, PARASITOIDS AND PREDATORS OF THE SPRUCE BARK BEETLE (*IPS TYPOGRAPHUS* L.) AND THEIR POTENTIAL USE IN BIOLOGICAL CONTROL – A REVIEW

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Abstract: The increasing occurrence of bark beetle outbreaks in European spruce forests has resulted in extensive economic losses over the last few decades. This damaging trend has created a demand for new environmentally friendly methods to keep populations of this forest pest at the lowest possible level. Biological control methods represent one option to prevent uncontrolled bark beetle population growth. *Ips typographus*, the European spruce bark beetle, is the target of a variety of natural enemies, which in natural conditions can effectively control its populations. This review summarizes our knowledge of species associated with the spruce bark beetle and presents the life cycle and virulence data for the most important species. Entomopathogenic fungi, parasitoids and predators are key biotic factors controlling bark beetle population dynamics in undisturbed spruce forest ecosystems and have good potential for use in biological control programs.

Key words: spruce bark beetle, *Ips typographus*, biological control, pathogens, parasitoids, predators

INTRODUCTION

Ips typographus (Coleoptera, Scolytidae) is an economically important pest attacking conifers, preferably Norway spruce. In endemic population levels spruce bark beetles attack weakened, fallen or snow broken trees, causing their death and eventual replacement with new growers (LIEUTIER 2007, SAUVARD 2007).

In healthy spruce forests, bark beetles occur in low abundance and play a sanitary role. They colonize weakened or dead trees and participate in the spruce forest rejuvenation. Natural events which seriously influence tree stands (dry seasons, wind storms, fire, fungal diseases) result in a large number of dead or damaged trees that are, without proper intervention, readily available for spruce bark beetle feeding and reproduction. Such circumstances usually lead to uncontrolled population increases and eventually mass population outbreaks within several years. When the adequate substrate during an outbreak is exhausted, beetles eventually attack healthy trees causing severe damage in the forest. Between the years 1990-2001, approximately 2,819,000 ha of Norway spruce (*Picea abies*) stands were attacked by *Ips typographus*, causing death to approximately 31,643,000 m³ of timber in Europe. In addition, a total

area of 4,821,000 ha of Norway spruce stands remains potentially threatened due to persistent abundant beetle population levels (GRÉGOIRE & EVANS 2007).

The population density of bark beetles in unaffected spruce forests is under the control of a complex of pathogens, parasitoids and predators preventing bark beetle population gradation. However, the controlling capacity of natural enemies is not sufficient when bark beetle outbreaks occur. During outbreaks, the populations of parasites increase as well, but the parasitism rate remains low (HOUGARDY & GRÉGOIRE 2001).

This paper provides a comprehensive overview of pathogens, predators and parasitoids which could be used for biological control of bark beetles. Biological control strategies, implementing spruce bark beetle natural antagonists in inundation and conservation biological control, present new possibilities for spruce bark beetle management and control without timber extraction and the resulting biodiversity and energy loss in the ecosystem.

1. Pathogens

Research on *Ips typographus* pathogens has nearly a 100-year history. A variety of pathogens associated with *I. typographus* were identified, including viruses (*Ips typographus* Entomopoxvirus ItEPV), bacteria (*Bacillus thuringiensis*, *B. sphaericus*, *Acinetobacter* spp., *Proteus vulgaris*, *Serratia liquefaciens*), fungi (*Beauveria bassiana*, *B. caledonica*, *B. brongniartii*, *Isaria farinosa*, *I. fumosorosea*, *Verticillium lecanii*), protozoans (*Metschnikowia typographi*, *Gregarina typographi*, *Malamoeba scolyti*, *Menzbieria chalcographi*), microsporidians (*Mattesia* sp., *Chytridiopsis typographi*, *Nosema typographi*, and *Unikaryon montanum*) (WEGENSTEINER 2007, MURATOĞLU et al. 2011), and nematodes (*Neoditylenchus*, *Sychnotylenchus*, *Parasitaphelenchus*, *Ektaphelenchus* and *Parasitorhabditis*) (MASSEY 1974), representing a fairly substantial repertoire of pathogens and possible candidates for spruce bark beetle biological control programs.

Viruses

There are a variety of viruses infecting invertebrates, but only *Ips typographus* entomopoxvirus (ItEPV) has been identified in bark beetles. This virus belongs to Entomopoxvirinae (family Poxviridae). Three genera can be distinguished inside this taxon: *Alphaentomopoxvirus* infecting beetles, *Betaentomopoxvirus* infecting butterflies and moths and *Gammaentomopoxvirus* infecting flies and mosquitoes (EBERLE et al. 2012). The group is characterized by a large linear molecule of double stranded DNA formed as brick-shaped virions that are occluded in a paracrystalline protein (spheroidin). The occluded body particles are known as spheroids (CORY & EVANS 2007).

Ips typographus entomopoxvirus (ItEPV) is the only virus infecting *I. typographus*. Adult beetles are infected by ingestion of frass containing spheroids from infected individuals in the gallery. The virus is initially localized in the anterior part of the intestine and spreads to posterior parts as infection progresses. Spheroids of the ItEPV are not present in other tissues of the host except the intestine. Infected

host cells are not deformed or otherwise modified in comparison to non-infected cells (WEGENSTEINER & WEISER 1994, YAMAN & BAKI 2011). The disease typically develops only in mature black beetles; light brown beetles do not contain inclusion bodies in their midgut epithelial cells. The virus creates focal points in field conditions (similar infection rates within a tree but marked differences between the trees); in some cases it was identified in up to 40 % of dead beetles in the galleries (WEISER et al. 2000). Although there are records of infection rates of the virus in populations of beetles, information about virus induced mortality is controversial. The mechanism bringing about host mortality is still unknown. Measurements of the spheroid size indicate that there may be differences among isolates from various geographic regions. These differences may influence pathogenicity and different insecticidal effects of the virus varieties can be expected (YAMAN & BAKI 2011). Although the prevalence of the virus in the natural population is generally low, exceptions were documented. The abundance of ItEPV found in *I. typographus* population in one Austrian site reached as high as 21.3 % (HAIDLER et al. 2003).

Bacteria

Entomopathogenic bacteria are frequently used in biological control programs against insect pests (LACEY et al. 2001, HAJEK & BAUER 2007).

The most common spore-forming bacteria employed in biological control of insects, *Bacillus thuringiensis* (Bt), was also isolated from the spruce bark beetles. Nevertheless, field trials with Bt var. *tenebrionis* sprayed on the spruce logs prior to beetle colonisation did not result in any significant reduction of tested bark beetle populations in the field (WEGENSTEINER 2007).

Research on bacterial communities associated with bark beetles which could lead to new potential biocontrol agents has been very scarce. MURATOĞLU et al. (2011) isolated 8 bacteria from live and dead *I. typographus* beetles. Of these, 5 caused mortality in healthy beetles under laboratory conditions (*Bacillus sphaericus*, two *Acinetobacter* spp., *Proteus vulgaris*, and *Serratia liquefaciens*). However, only *S. liquefaciens* demonstrated some potential for biocontrol, causing 53.3% mortality among the infected adults of *I. typographus* (MURATOĞLU et al. 2011).

Protozoa

Gregarina typographi, *Menzbieria typographi* (Apicomplexa: Gregarinidae) and *Malamoeba scolyti* (Sarcomastigophora: Amoebidae) are protozoan pathogens found in *Ips typographus* (WEGENSTEINER 2007).

Gregarines are defined by the presence of a unique organelle called apical complex. Sporozoites released from oocyst in intestine either penetrate the host peritrophic membrane and invade into intestinal epithelium or migrate through the epithelium to Malpighian tubes, gonads, or fat body where they transform to trophozoites (VALIGUROVÁ 2007).

The life cycle of *Gregarina typographi* is split into several stages (LUKÁŠOVÁ & HOLUŠA 2012). Bark beetles are infected inside the bark by ingestion of *G. typographi* oocysts in the gallery system. After ingestion, the oocyst collapses,

releasing sporozoites infecting the intestine lumen. Sporozoites attach to host intestine cells between microvilli by the epimerite. Detachment of the mature trophozoites is through epimerite retraction into the protomerite (VALIGUROVÁ et al. 2009). Trophozoites can even reattach in search for adequate cells (VALIGUROVÁ 2012). Free detached trophozoites (gamonts) can copulate in host intestine. Two haploid gamonts form syzygium which undergoes gamogonia and creates gametocysts (LUKÁŠOVÁ & HOLUŠA 2011). Gametocysts undergo development through gamogony and then leave the intestine with the feces. The dynamics of *G. typographi* in *I. typographus* was studied by several authors and marked variations in prevalence was observed between different years (WEGENSTEINER & WEISER 2004). MICHALKOVÁ et al. (2012) detected considerable variability in the number of infected beetles (33.3 to 97.5%) in *I. typographus* at the same site in one season. The developmental cycle of gregarines may be carried out several times during the feeding of the beetles in the bark (LUKÁŠOVÁ & HOLUŠA 2011).

Another gregarine species, *Menzbieria chalcographi* (Apicomplexa, Neogregarinida), attacks the fat body of *I. typographus* producing numerous spores, which are released after the death of the beetle in the gallery (WEISER et al. 2000). It is a rare pathogen, usually present with prevalence as low as 0.7 % (WEGENSTEINER & WEISER 2004).

Malamoeba scolyti (Rhizopoda, Amoebidae) was first discovered in *Dryocoetes autographus* (Ratzeburg) (WEGENSTEINER 2007). *M. scolyti* develops intracellularly in the midgut epithelium and Malpighian tubes (HÄNDEL et al. 2003). Infection is peroral by the cysts in the gallery environment (KIRCHHOFF & FÜHRER 1990). Prevalence is generally low but in some cases infection rates reach high values. MICHALKOVÁ et al. (2012) found a high infection rate (64.7%) in *I. typographus* at one of the studied sites in Slovakia but provided no explanation for this phenomenon. *M. scolyti* infection results in hindered movement, progressive mobility difficulties, absence of flight activity and eventual death of the beetles (KIRCHHOFF & FÜHRER 1990).

Fungi

Entomopathogenic fungi constitute a large group of approximately 700 species (ROBERTS 1989). Many studies were focused on exploring their potential in biological control (e.g. WEGENSTEINER 2007). Entomopathogenic fungi are either host specific or with a wide host range varying in infection rates (SHAH & PELL 2003, Shahid et al. 2012). Various strains and cryptic species within entomopathogenic fungi can be identified by differences in some physiological traits as well as by molecular markers (BIDOCHKA et al. 2001). Several species are already used in biological control programs of insect pests around the world, particularly those from genera *Metarhizium*, *Beauveria*, *Verticillium*, *Isaria* and *Entomophthora* (SHAH & PELL 2003). The virulence of *B. bassiana*, *I. farinosa*, *M. anisopliae* and *V. lecanii* was tested on *I. typographus* with significant mortality effect, indicating their suitability for biological control against this forest pest (POPA et al. 2012).

Infection generally begins by adhesion of the spores on the surface of the insect and is followed by germination and penetration of the cuticle of its host through mechanical and enzymatic mechanisms. Mechanical penetrational force is generated

by an appressorium - a specialized structure developed for penetration through the cuticle employing turgor pressure to a narrow peg (SHAHID et al. 2012). After successful penetration the fungus produces hyphal bodies which are distributed passively in the hemolymph and invade other host tissues. The fungus depletes nutrients present in the host's hemolymph and fat body resulting in the death of the insect. The fungus then, under humid conditions, starts the saprophytic stage of its development, emerges out of the body and produces aerial conidia on the exterior surface of the dead host.

A complex of biotic and abiotic factors affects the ability of the fungi to survive, propagate, infect and kill their host. Solar radiation, temperature and relative humidity, including water, are among the most important parameters affecting germination, vegetative growth and viability of entomopathogenic fungi. Entomopathogenic fungi generally require high humidity (92-100 %) to germinate and sporulate (ZIMMERMANN 2007a,b). Ultraviolet B (280-320 nm) and ultraviolet A (320-400 nm) radiation are the most lethal (BRAGA et al. 2001). Entomopathogenic fungi have a great tolerance to sub-zero temperatures but the threshold for high temperatures over long time periods is only 40°C (WRAIGHT et al. 2007).

Although the entomopathogenic fungi are able to kill all life stages of bark beetles if they have access to their cuticle, they do not generally interfere with immature stages (eggs, larvae, pupae, callow adults) during their development in galleries in fresh bark (WEGENSTEINER 2007).

Beauveria bassiana is used in biological control of a variety of insect pests (SHAH & PELL 2003). It has a wide host range and is distributed in all main biogeographic regions, where it is an integral part of microbial flora and regulates various insect populations in natural environments by epizootics (FENG et al. 1994, MEYLING & EILENBERG 2007). *B. bassiana* is a very variable species; enzymatic and DNA characteristics among strains as well as virulence and pathogenicity to different arthropods vary considerably (STEINWENDER et al. 2010). During its development in insects *B. bassiana* produces a number of secondary metabolites (beauvericin, bassianin, bassianolide, beauverolides, beauveriolides, tenellin, oosporein, oxacid acid and bassiacridin) which reduce the immune reaction of the host and inhibit growth of potential competitors (ZIMMERMANN 2007a). Prevalence depends on dosage and temperature but other factors like humidity are also of significant importance. Optimum temperature for the growth of *B. bassiana* is 23-28 °C, minimum 5-10 °C and maximum 30-38 °C, depending on the isolates. Temperature of 50 °C for 10 min is lethal for the spores (ZIMMERMANN 2007a). Humidity is important for germination of spores and sporulation after death of the host. Generally, conidia of *B. bassiana* require high relative humidity (92-100 %) to germinate, but insect infections were observed at relative humidities as low as 60-70 %, presumably as a result of high local relative humidity in the microhabitat on the insect cuticle or foliage (ZIMMERMANN 2007a).

Inundative release of *I. typographus* infected by *B. bassiana* by the contaminated pheromone traps resulted in high infection rates (VAUPEL & ZIMMERMANN 1996). Efficiency of the pathogen transfer and consecutive mortality of the beetles was

highly significant (KREUTZ et al. 2004a). KREUTZ et al. (2004b) tested a commercial *B. bassiana* suspension (Boverol[®]) and several isolates on *Ips typographus* adults and obtained 99-100% mortality of the bark beetles after 7 days. Variations of humidity tested with the commercial product showed some differences but the tested range (40-100% RH) was well tolerated by the fungus. *B. bassiana* also showed high pathogenicity in *Scolytus scolytus* (F.), *Pityogenes chalcographus* (L.) and *Ips sexdentatus* (Boerner) (POPA et al. 2012).

Isolation of an aggressive native strain incorporated in a bioassay infecting *I. typographus* allows us to avoid the introduction of exotic strains (LANDA et al. 2001, REAY et al. 2008). Targeted application of *B. bassiana* spores by pheromone traps reduces the negative impact on beneficial insects. Application of the conidia suspension on tree stems (JAKUŠ & BLAŽENEC 2011) is also an alternative but there is a risk of negative effects to non-target organisms.

Metarhizium anisopliae is a species with a long history of use in the biocontrol of insect pests. It is a typical soil-borne fungus very sensitive to UV light; it can be found on insects as well as in the soil worldwide. *M. anisopliae* has a narrower host range compared to *B. bassiana* and infects particularly Coleoptera and soil-dwelling pests, although it was isolated from most insect orders and even mites, ticks and amphipods (ZIMMERMANN 2007b). It is a mesophilic fungus with a temperature range generally between 15-35 °C, with an optimum range for germination and growth between 25-30 °C (ZIMMERMANN 2007b), although cold-active and heat-tolerant isolates were reported. Many isolates and genetic groups show some host-insect preferences and are often more specific (even species-specific) in the field than under laboratory conditions (ZIMMERMANN 2007b). Infection is very similar to *B. bassiana*. The fungus produces several metabolites, including destruxins, cytochalasins, and swainsonine in culture and *in vivo*. More bioactive compounds were isolated from culture broth (insecticidal antibiotics hydroxyfungierins and others). Its persistence in soil is higher than that of *B. bassiana* (VÄNNINEN et al. 2000) and may reach 3-4 years.

Isaria fumosorosea species complex and *I. farinosa* (formerly *Paecilomyces fumosoroseus* and *P. farinosus*) are entomopathogenic soil-borne fungi with worldwide distribution in temperate and tropical zones and have a relatively narrow host range compared to *B. bassiana* or *M. anisopliae*. The fungus was isolated from Lepidoptera, Homoptera, Hemiptera (particularly whiteflies), Coleoptera, Hymenoptera, Diptera, Thysanoptera, spiders, ticks and mites (ZIMMERMANN 2008). *I. farinosa* has been isolated from a range of bark beetle species, including *I. typographus* (WEGENSTEINER 2007). Insects are often cross-infected with *B. bassiana* and *I. fumosorosea* (mixed infection). The fungi produce toxins such as beauvericin, pyridine-2,6-dicarboxylic acid, beauverolides, dipicolinic acid, and others (ZIMMERMANN 2008). *I. farinosa* can grow in a temperature range between 2-5 °C and 30-32 °C with an optimum for germination and growth between 19 and 22.5 °C (ZIMMERMANN 2008). *I. fumosorosea* has a wider temperature tolerance with an optimum at 20-30 °C with marked differences between isolates. *I. fumosorosea* is more sensitive to solar radiation than *M. anisopliae* or *B. bassiana* (FARGUES et al.

1997). Some pathogenicity against non-target organisms (primarily predators and parasitoids) was reported in laboratory bioassays (ZIMMERMANN 2008).

Microsporidia

Microsporidia represent a unique group of entomopathogens. The effect on the host is chronic, rather than acute. Microsporidia are single-celled organisms that reproduce in living cells (intracellular parasites). The infective form is an environmental spore consisting of a proteinaceous exospore and an inner plasma membrane (plasmalemma) surrounding the sporoplasm. Protein-chitin matrix separates exospore and plasmalemma. At the end of the spore is the polar filament coiled within the sporoplasm which is projected from the spore in the process of host cell infection (SOLTNER & BECNEL 2007). *Nosema locustae* is the only microsporidian used in biological control against grasshoppers and crickets thus far (SOLTER & BECNEL 2007).

Chytridiopsis typographi is a microsporidian intracellular parasite recorded from several bark beetle species (TAKOV et al. 2010). This species requires no intermediate hosts and its life cycle is completely tied to bark beetles. Infection occurs after ingestion of the spore in the gallery of the host or by transovarial transmission (LUKÁŠOVÁ & HOLUŠA 2011). Development is intracellular and occurs in the midgut epithelium, progressing to gonads, of bark beetles. Spores are characterized with a polar filament, which allows infection of *Ch. typographi* by sporoplasm transmission to the cytoplasm of the cell in the intestine epithelium (LUKÁŠOVÁ & HOLUŠA 2011). It produces two types of spores, for dispersal inside the host (thin walled cysts) and within the host population (thick walled cysts - environmental spores). Abundance of *Ch. typographi* varies from 1 to 60% in *I. typographus* populations (WEGENSTEINER & WEISER 2004). *Ch. typographi* is characteristic for its fluctuations throughout the years (WEGENSTEINER et al. 1996, HÄNDEL et al. 2003). The pathogen destroys midgut epithelium, influences the fat body production, reduces host dispersion (WEGENSTEINER et al. 2010) and affects survival when hibernating (ANDERBRANT 1988).

Unikaryon montanum and *Nosema typographi* are microsporidian pathogens found in spruce bark beetles. Prevalence of these pathogens is generally low, around 1% for both species (HÄNDEL et al. 2003). Both pathogens attack fat body and Malpighian tubes, and produce spores which are secreted within the feces in the gallery (LUKÁŠOVÁ & HOLUŠA 2011).

Nematodes

The majority of nematodes of bark beetles are obligatory parasites. Some species associated with bark beetles are phoretic and are carried (e.g. in small cocoons) beneath the elytrae of the adults. Phoretic nematodes associated with *I. typographus* are representatives of superfamilies Aphelenchoidea (*Ektaphelenchus typographi* (Fuchs), *Cryptaphelenchus typographi*, *Bursaphelenchus typographi*, *B. eidmanni* and *Tylaphelenchus christinae*), Rhabditoidea (*Mikoletzkyia*, *Neocephalobus*, *Diplogasteroides*, *Rhabdontolamius*, *Cylindrocorypus* and *Acrostichus*) and

Tylenchoidea (*Neoditylenchus* and *Sychnotylenchus*) (MASSEY 1974, ZHAO 2006). Parasitic nematodes attacking bark beetles belong mainly to two following suprefamilies. Genera *Parasitylenchus* and *Contortylenchus*, both with similar life history, are ordered to Neotylenchoidea. Infected beetles deposit the immature form of the nematode into their breeding system where they develop to mature forms and copulate outside the host. Impregnated females immediately penetrate through the cuticle into the body cavity of the host where they lay offspring. Offspring are deposited in the body cavity of the host where they develop, penetrating to the intestine, from where they are excreted with the feces to complete the cycle inside the gallery. Aphelenchoidea (with *Parasitaphelenchus* and *Ektaphelenchus*) and Rhabditoidea (*Parasitorhabditis*) are accidental internal parasites of bark beetles (MASSEY 1974).

2. Parasitoids

Parasitoids pose a transitive stage between parasites and predators. They are organisms associated with a particular developmental stage of other arthropods and kill their hosts at the end of their development. Four parasitoid guilds are recognized: 1. egg parasitoids, 2. egg-larval endoparasitoids, 3. larval ectoparasitoids and 4. adult endoparasitoids (KENIS et al. 2007). Most of the parasitoids of Scolytidae belong to the larval and adult endoparasitoids guild. Larval ectoparasitoids locate their hosts from the surface of the bark. They either enter the galleries (cryptoparasitoid species) or attack the hosts through the bark directly. The species that belong to the second strategy have a long ovipositor which they use to penetrate the bark to inject venom and lay eggs in the host. As soon as the egg is laid, the development of the host is immediately paralyzed by the venom. The development of parasitoid preimaginal stages is fast, they usually overwinter as prepupae or pupae in the host gallery. In some cases the female stays with the progeny in order to protect the breeding site from competitors and hyperparasitoids and/or to provide further paralysis of the host (HOLECOVÁ 2012). Adult endoparasitoids oviposit into adults of various ages directly, locating them on the bark surface. Parasitized beetles are able to function normally after the inoculation but their fecundity is significantly reduced. The host is eventually killed by the parasitoid in the gallery. Adult endoparasitoids are more specialized compared to larval ectoparasitoids, more fecund and with a longer developmental time. Host location mechanism of the parasitoids is of essential interest in the field of biological control strategy improvements (KENIS et al. 2007). Many parasitoids and predators are attracted to frass and pheromones of adults or larvae of bark beetles. These semiochemicals also interfere with oxygenated monoterpenes of the host tree. Parasitoids are attracted to plant volatiles released by infestation-associated yeasts and fungi and/or the vascular tissue of the host tree (PETTERSSON 2001a). There are only a few compounds from the chemical spectrum of infested tree logs that act as main orientation cue for parasitoids. These chemicals are specific to the infested logs and occur in prevalence when logs contain mature larvae (PETTERSSON et al. 2001, PETTERSSON 2001b). For example, *Roptrocercus* spp. and *Rophalicus tutela* (Walker) respond mainly to the oxygenated monoterpenes

that indicate damaged conifers (PETTERSSON 2001a, b). These molecules can be used as synthetic attractants for bark beetle parasitoids. This can enhance the efficiency of the parasitoid impact on the bark beetle population when applied in the field using biological control strategy (PETTERSSON et al. 2001).

Several species have been identified as parasitoids of *I. typographus* and a comprehensive list is presented by KENIS et al. (2007). The most significant larval ectoparasitoids of the spruce bark beetle are *Coeloides bostrichorum* (Giraud), *Dendrosoter middendorfi* (Ratzeburg), *Rhopalicus tutela*, *Dinotiscus eupterus* (Walker), *Heydenia pretiosa* (Forster), *Roptrocerus mirus* (Walker), *R. xylophagorum* (Ratzeburg), *Eurytoma arctica* (Thomson), *E. blastophagi* (Hedqvist) and *E. morio* (Boheman). Adult endoparasitoids include *Cosmophorus klugii* Ratzeburg, *C. regius* Niezabitowski, *Ropalophorus clavicornis* (Wesmael), *Tomicobia seitneri* (Ruschka) and *Mesopolobus typographi* (Ruschka). In addition *R. xylophagorum* has a cryptoparasitoid behavior (entering host galleries) whereas *M. typographi* is characterized as essentially hyperparasitoid species (attacks other parasitoid species). Population dynamics of parasitoids generally follow the same pattern as bark beetle populations without any appreciable time lag (WERMELINGER et al. 2013). Stand properties, openness of the habitat, host availability, the frequency and the extent of environmental disturbances influence the abundance of parasitoids and predators (WESLIEN & SCHROEDER 1999, WERMELINGER et al. 2013). Forest management influences parasitoids and predators essentially but has less impact on bark beetle populations. Management of forests affected by environmental disaster needs to be balanced and part of fallen trees should be left on site as a reservoir of parasitoids population (WESLIEN & SCHROEDER 1999).

3. Predators

Predators are organisms actively tracing their prey on which they feed during their larval and/or adult instars. They are mainly generalists preying on a wide range of species. Prey location is through the host pheromones to which they respond. Attractiveness of prey pheromones depends on composition of tree volatiles (ERBILGIN & RAFFA 2001, KENIS et al. 2007). Among predators associated with bark beetles, coleopteran species are the most important. Cleridae and Rhizophagidae are recognized as having a great impact on bark beetle population dynamics (KENIS et al. 2007). Clerid beetles prey on bark beetle adults as well as their larvae. The adults locate their prey by pheromones of the bark beetles mixed with the volatiles from the attacked tree. They occur at the infested tree shortly after the bark beetle attack. Bark beetles are captured and consumed directly on the bark surface. Mating and oviposition of clerids occur on the bark surface as well. Newly hatched clerid larvae enter the galleries where they feed on bark beetle brood (KENIS et al. 2007, REEVE 1997). *Thanasimus formicarius* (L.) is a major natural enemy of *Tomicus piniperda* (L.) and *I. typographus* in Eurasia (KENIS et al. 2007). The estimated value of *T. formicarius* prey consumption is three bark beetles per day for adults and approximately fifty bark beetle larvae for one larva of the predator for its whole larval period (DIPPEL et al. 1997). *I. typographus* population decline due to *T.*

formicarius predation was in some cases estimated to be 45 % (WESLIEN & REGNANDER 1992). The fecundity of *T. formicarius* under laboratory conditions was on average 162 eggs per female (range: 71-307) indicating a great potential for biocontrol. However, field studies indicated that high densities of adult beetles do not necessarily result in a lower number of emerged bark beetles and some competition between *T. formicarius* individuals probably takes place (WESLIEN & REGNANDER 1992). In the laboratory, the predation rate was estimated to be, on average, 0.86 *I. typographus* per pair of *T. formicarius* per day (WESLIEN & REGNANDER 1992). The introduction of this predator still requires further research on mass-rearing techniques and environmental conditions correlations (REEVE et al. 2003).

Larvae of predatory flies *Medetera* are commonly found in the galleries feeding on larvae of bark beetles (MAMONTOV 2009). In the study of WESLIEN & REGNANDER (1992) exclusion of natural enemies (primarily *T. formicarius* and *Medetera* spp.) by caging of the spruce logs resulted in a 5-fold increase in bark beetle productivity.

Relationship between bark beetles and mites can range from mutualistic to parasitic, however the influence of associated mites on *I. typographus* are not sufficiently explored. *Iponemus* spp. and *Paracarophenax* spp. are predators of bark beetle eggs, *Pyemotes* spp. and *Digamasellus* spp. feed on larvae and pupae of the bark beetles. Adult beetles are not parasitized or consumed by whatever mite is associated (KENIS et al. 2007).

Woodpeckers (mainly *Picoides* spp.) are of significance to natural regulation of bark beetle populations. *Picoides tridactylus* (L.) is a species which can influence population size of bark beetles, however their abundances in spruce forests remain low (KENIS et al. 2007).

CONCLUSIONS

Effective control of *I. typographus* populations is difficult due to its hidden biology. Except for a short period of dispersal flight, the beetles and their progeny spend their entire life under the bark, inside spruce phloem. Conventional control methods which are used to suppress bark beetle outbreaks include sanitary cutting, clearing windthrows, use of tree traps treated by insecticides and pheromone traps. Although very effective, conventional methods of bark beetle control pose environmental risks. The use of insecticides is controversial, not only because of its negative effect on complex forest ecosystems, but also due to its limited effect, since all preimaginal stages as well as the majority of adults are protected by the tree bark. Sanitary cutting and clearing of fallen trees cause a local diversity decline and affect the food chain in the forest ecosystem as well.

Biological control is defined as the use of living organisms to suppress the population density or impact of a specific pest. This strategy uses natural pathogens, parasitoids and predators to reduce populations of desired target organism. Biological agents used in biological control are selective and preferably attack the target pest. In stable ecosystems pathogens, parasitoids and predators promote effective reduction of pest populations and inhibit its growth.

This review demonstrates that a variety of species associated with *I. typographus* have the potential to be used as effective biological agents to prevent uncontrolled growth of its populations and keep it under an economically damaging threshold. Protozoans and microsporidians reduce fitness, fertility and life span of infested individuals. They are important indicators in the evaluation of pest population health status and can be considered for biological control programs.

Distinct progress was achieved in the use of entomopathogenic fungi, mainly *Beauveria bassiana*, to control bark beetles. The virulence of isolated native strains is high and its spores can be easily produced and distributed. The distribution of pathogens via pheromone traps increases targeted application and substantially reduces any negative effect on the forest ecosystem. Infected beetles become carriers and further spread the pathogens in bark beetle populations.

Parasitoids and predators can significantly reduce their host populations. They are one of the main mortality factors of herbivore insects, attacking mainly mid/late larval stages. *R. xylophagorum* was applied in Australia against introduced bark beetle *I. grandicollis* (Eichhoff) (SAMSON & SMIBERT 1986). Management of bark beetle populations utilizing both parasitoids and synthetic attractants to enhance the parasitoid efficiency in host location can be an integral part of conservation biological control strategy (PETTERSSON et al. 2001).

Acceleration of climatic changes in recent years increased the frequency of disturbances and resulted in serious damage of spruce stands in Europe, followed by outbreaks of bark beetle populations. This situation underlines the importance of prevention and keeping bark beetle populations at the lowest possible level. Biological control strategies offer new environmentally friendly methods in prevention and control of bark beetle outbreaks.

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REFERENCES

- ANDERBRANT O. 1988. Survival of parent and brood adult bark beetles, *Ips typographus*, in relation to size, lipid content and re-emergence or emergence day. *Physiological Entomology* 13: 121-129.
- BIDOCHKA M.J., KAMP A.M., LAVENDER T.M., DEKONING J., DE CROOS J.N.A. 2001. Habitat association in two genetic groups of the insect-pathogenic fungus *Metarhizium anisopliae*: Uncovering cryptic species? *Applied and Environmental Microbiology* 67: 1335-1342.
- BRAGA G.U.L., FLINT S.D., MILLER C.D., ANDERSON A.J., ROBERTS D.W. 2001. Both solar UVA and UVB radiation impair conidial curability and delay germination in the entomopathogenic fungus *Metarhizium anisopliae*. *Photochemistry and Photobiology* 74: 734-739.
- CORY J.S., EVANS H.F. 2007. Viruses, pp. 149-174. In: Lacey L.A., Kaya H.K. (eds) *Field manual of techniques in invertebrate pathology*. Springer, Dordrecht.
- DIPPEL C., HEIDGER C., NIKOLAI, V., SIMON, M. 1997. The influence of four different predators on bark beetles in European forest ecosystems. *Entomologia Generalis* 21: 161-175.

- EBERLE K.E., WENNMANN J.T., KLEESPIES R.G., JEHL J.A. 2012. Basic techniques in insect virology, pp. 15-74. In: Lacey L.A., Kaya H.K. (eds) *Manual of techniques in invertebrate pathology*. Academic Press, London, Waltham, San Diego.
- ERBILGIN N., RAFFA F.K. 2001. Modulation of predator attraction to pheromones of two prey species by stereochemistry of plant volatiles. *Oecologia* 127: 444-453.
- FARGUES J., ROUGIER M., GOUJET R., SMITS N., COUSTERE C., ITIER B. 1997. Inactivation of conidia of *Paecilomyces fumosoroseus* by near-ultraviolet (UVB and UVA) and visible radiation. *Journal of Invertebrate Pathology* 69: 70-78.
- FENG M.G., POPRAWSKI T.J., KHACHATOURIANS G.G. 1994. Production, formulation and application of the entomopathogenic fungus *Beauveria bassiana* for insect control: Current status. *Biocontrol Science and Technology* 4: 3-34.
- GRÉGOIRE J.-C., EVANS F.H. 2007. Damage and control of BAWBILT organisms an overview, pp. 19-37. In: Lieutier F., Day K.R., Battisti A., Grégoire J.-C., Evans, H.F. (eds) *Bark and wood boring insects in living trees in Europe, a synthesis*. Springer, Dordrecht.
- HAIDLER B., WEGENSTEINER R., WEISER J. 2003. Occurrence of microsporidia and other pathogens in associated living spruce bark beetles (Coleoptera: Scolytidae) in an Austrian forest. *IOBC-WPRS Bulletin* 26: 257-260.
- HAJEK A.E., BAUER L.S. 2007. Microbial control of wood-boring insects attacking forest and shade trees, pp. 505-526. In: Lacey L.A., Kaya H.K. (eds) *Field manual of techniques in invertebrate pathology*. Springer, Dordrecht.
- HÄNDEL U., WEGENSTEINER R., WEISER J., ŽIŽKA Z. 2003. Occurrence of pathogens in associated living bark beetles (Col., Scolytidae) from different spruce stands in Austria. *Journal of Pest Science* 76: 22-32.
- HOLECOVÁ M. 2012. *Parazitoidy a ich životné stratégie*. AQ-BIOS, Bratislava, 47 pp.
- HOUGARDY E., GRÉGOIRE C.-J. 2001. Bark-beetle parasitoids population surveys following storm damage in spruce stands in the Vosges region (France). *Integrated Pest Management Reviews* 6: 163-168.
- JAKUŠ R., BLAŽENEC M. 2011. Treatment of bark beetle attacked trees with entomopathogenic fungus *Beauveria bassiana* (Balsamo) Vuillemin. *Folia Forestalia Polonica* 53: 150-155.
- KENIS M., WERMELINGER B., GRÉGOIRE C.-J. 2007. Research on parasitoids and predators of Scolytidae – a review, pp. 237-290. In: Lieutier F., Day K.R., Battisti A., Grégoire J.-C., Evans, H.F. (eds) *Bark and wood boring insects in living trees in Europe, a synthesis*. Springer, Dordrecht.
- KIRCHHOFF J.-F., FÜHRER E. 1990. Experimentelle Analyse der Infektion und des Entwicklungszyklus von *Malamoeba scolyti* in *Dryocoetes autographus* (Coleoptera: Scolytidae). *Entomophaga* 34: 537-544.
- KREUTZ J., VAUPEL O., ZIMMERMANN G. 2004a. Efficacy of *Beauveria bassiana* (Bals.) Vuill. against the spruce bark beetle, *Ips typographus* L., in the laboratory under various conditions. *Journal of Applied Entomology* 128: 384-389.
- KREUTZ J., ZIMMERMANN G., VAUPEL O. 2004b. Horizontal transmission of the entomopathogenic fungus *Beauveria bassiana* among the spruce bark beetle, *Ips typographus* (Col., Scolytidae) in the laboratory and under field conditions. *Biocontrol Science and Technology* 14: 837-848.
- LACEY A.L., FRUTOS R., KAYA K.H., VAILS P. 2001. Insect pathogens as biological Control Agents: Do They have a Future? *Biological Control* 21: 230-248.
- LANDA Z., HORŇÁK P., OSBORNE L., NOVÁKOVÁ A., BURSOVÁ E. 2001. Entomogenous fungi associated with spruce bark beetle *Ips typographus* L. (Coleoptera, Scolytidae) in the Bohemian Forest. *Silva Gabreta* 6: 259-272.
- Lieutier F. 2007. Host resistance to bark beetles and its variations, pp. 135-180. In: Lieutier F., Day K.R., Battisti A., Grégoire J.-C., Evans, H.F. (eds) *Bark and wood boring insects in living trees in Europe, a synthesis*. Springer, Dordrecht.
- LUKÁŠOVÁ K., HOLUŠA J. 2011. *Gregarina typographi* (Eugregarinoida: Gregarinidae) in the bark beetle *Ips typographus* (Coleoptera: Curculionidae): Changes in infection level in the breeding system. *Acta Protozoologica* 50: 311-318.
- LUKÁŠOVÁ K., HOLUŠA J. 2012. Patogeny lýkožroutů rodu *Ips* (Coleoptera: Curculionidae: Scolytinae): review. *Zprávy Lesnického Výzkumu* 57: 230-240.

- MAMONTOV S.N. 2009. Raspredelenie po stvolu dereva koroeda-tipografa (*Ips typographus*, Coleoptera, Scolytidae) i ego entomofagov [Distribution of the bark beetle (*Ips typographus*) and its entomophages along tree trunk]. *Zoologicheskij Zhurnal* 88: 1139-1145.
- MASSEY C.L. 1974. *Biology and taxonomy of nematode parasites and associates of bark beetles in the United States*. U.S. Department of Agriculture, Washington, 233 pp.
- MICHALKOVÁ V., KRASCENITSOVÁ E., KOZÁNEK M. 2012. On the pathogens of the spruce bark beetle *Ips typographus* (Coleoptera: Scolytinae) in the Western Carpathians. *Biologia* 67: 217-221.
- MEYLING V.N., EILENBERG J. 2007. Ecology of the entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae* in temperate agroecosystems: Potential for conservation biological control. *Biological Control* 43: 145-155.
- MURATOĞLU H., SEZEN K., DEMIRBAG Z. 2011. Determination and pathogenicity of the bacterial flora associated with the spruce bark beetle, *Ips typographus* (L.) (Coleoptera: Curculionidae: Scolytinae). *Turkish Journal of Biology* 35: 9-20.
- PETTERSSON E.M. 2001a. Volatile attraction of three Pteromalid parasitoids attacking concealed spruce bark beetle. *Chemoecology* 11: 89-95.
- PETTERSSON E.M. 2001b. Volatiles from potential hosts of *Rhopalicus tutela* a bark beetle parasitoid. *Journal of Chemical Ecology* 27: 2219-2231.
- PETTERSSON E.M., BIRGERSSON G., WITZGALL P. 2001. Synthetic attractants for the bark beetle parasitoid *Coeloides bostrichorum* Giraud (Hymenoptera: Braconidae). *Naturwissenschaften* 88: 88-91.
- POPA V., DÉZIEL E., LAVALLÉE R., BAUCE E., GUERTIN C. 2012. The complex symbiotic relationships of bark beetles with microorganisms: a potential practical approach for biological control in forestry. *Pest Management Sciences* 68: 963-975.
- REAY D.S., BROWNBIDGE M., CUMMINGS J.N., NELSON L.T., SOUFFRE B., LIGNON C., GLARE R.T. 2008. Isolation and characterisation of *Beauveria* spp. Associated with exotic bark beetles in New Zealand *Pinus radiata* plantations forests. *Biological Control* 46: 484-494.
- REEVE D.J. 1997. Predation and bark beetle dynamics. *Oecologia* 112: 48-54.
- REEVE D.J., ROJAS M.G. & MORALES-RAMOS A.J. 2003. Artificial diet and rearing methods for *Thanasimus dubius* (Coleoptera: Cleridae), a predator of bark beetles (Coleoptera: Scolytidae). *Biological Control* 27: 315-322.
- ROBERTS W.D. 1989. World picture of biological control of insects by fungi. *Mem. Inst. Oswaldo Cruz* 84: 89-100.
- SAMSON P.R., SMIBERT J. 1986. Preliminary studies on the efficacy and establishment of *Roptrocerus xylophagorum* (Hym.: Torymidae), a parasitoid of *Ips grandicollis* (Col.: Scolytidae), in Australia. *Entomophaga* 31: 173-298.
- SAUVARD D. 2007. General biology of bark beetles, pp. 63-88. In: Lieutier F., Day K.R., Battisti A., Grégoire J.-C., Evans H.F. (eds) *Bark and wood boring insects in living trees in Europe, a synthesis*. Springer, Dordrecht.
- SHAH A.P., PELL K.J. 2003. Entomopathogenic fungi as biological control agents. *Applied Microbiology and Biotechnology* 61: 413-423.
- SHAHID A.A., RAO Q.A., BAKHSH A., HUSAIN T. 2012. Entomopathogenic fungi as biological controllers: new insights into their virulence and pathogenicity. *Archives of Biological Sciences* 64: 21-42.
- SOLTER L.F., BECNEL J.J. 2007. Entomopathogenic microsporidia, pp. 199-222. In: Lacey L.A., Kaya H.K. (eds) *Field manual of techniques in invertebrate pathology*. Springer, Dordrecht.
- STEINWENDER M.B., KRENN W.H., WEGENSTEINER R. 2010. Different effects of the insectpathogenic fungus *Beauveria bassiana* (Deuteromycota) on the bark beetle *Ips sexdentatus* (Coleoptera: Curculionidae) and on its predator *Thanasimus formicarius* (Coleoptera: Cleridae). *Journal of Plant Diseases and Protection* 117: 33-38.
- TAKOV D., PILARSKA D., WEGENSTEINER R. 2010. List of Protozoan and Microsporidian Pathogens of Economically Important Bark Beetle Species (Coleoptera: Curculionidae: Scolytinae) in Europe. *Acta Zoologica Bulgarica* 62: 201-209.

- VALIGUROVÁ A. 2007. *Comparative morphology of developmental stages of gregarines and cryptosporidia with an emphasis on host-parasite interactions*. Ph.D. Thesis. Masaryk University, 293 pp.
- VALIGUROVÁ A. 2012. Sophisticated adaptations of *Gregarina cuneata* (Apicomplexa) feeding stages for epicellular parasitism. *PLoS ONE* 7: e42606.
- VALIGUROVÁ A., MICHALKOVÁ V., KOUDELA B. 2009. Eugregarine trophozoite detachment from the host epithelium via epimerite retraction: Fiction or fact? *International Journal of Parasitology* 39: 1235-1242.
- VÄNNINEN, I., TYNI-JUSLIN J., HOKKANEN H. 2000. Persistence of augmented *Metarhizium anisopliae* and *Beauveria bassiana* in Finnish agricultural soils. *Biocontrol* 45: 201-222.
- VAUPEL O., ZIMMERMANN G. 1996. Orientierende Versuche zur Kombination von Pheromonfallen mit dem insectenpathogenen Pilz *Beauveria bassiana* (Bals.) Vuill. gegen die Borkenkäferart *Ips typographus* L. (Coleoptera, Scolytidae). *Journal of Pest Science* 69: 175-179.
- WEGENSTEINER R. 2007. Pathogens in bark beetles, pp. 291-314. In: Lieutier F., Day K.R., Battisti A., Grégoire J.-C., Evans, H.F. (eds) *Bark and wood boring insects in living trees in Europe, a synthesis*. Springer, Dordrecht.
- WEGENSTEINER R., WEISER J. 1994. A new entomopoxvirus in the bark beetle *Ips typographus* (Coleoptera: Scolytidae). *Journal of Invertebrate Pathology* 65: 203-205.
- WEGENSTEINER R., WEISER J. 2004. Annual variation of pathogens occurrence and pathogen prevalence in *Ips typographus* (Coleoptera, Scolytidae) from the BOKU University Forest Demonstration Centre. *Journal of Pest Science* 77: 221-228.
- WEGENSTEINER R., DEDRYVER A.-C., PIERRE S.-J. 2010. The comparative prevalence and demographic impact of two pathogens in swarming *Ips typographus* adults: a quantitative analysis of long term trapping data. *Agricultural and Forest Entomology* 12: 49-57.
- WEGENSTEINER R., WEISER J., FUHRER E. 1996. Observations on the occurrence of pathogens in the bark beetle *Ips typographus* L. (Col., Scolytidae). *Journal of Applied Entomology* 20: 199-204.
- WEISER J., PULTAR O., ŽIŽKA Z. 2000. Biological protection of forest against bark beetle outbreaks with poxvirus and other pathogens. Proceedings of the 12th Regional central European conference IUAPPA and 4th International conference on environmental impact assessment, September 11-14th, Prague; section B: 168-172.
- WERMELINGER, B., OBRIST M.K., BAUR H., JAKOBY O., DUELLI P. 2013. Synchronous rise and fall of bark beetle and parasitoid populations in windthrow areas. *Agricultural and Forest Entomology* 15: 301-309.
- WESLIEN J., REGNANDER J. 1992. The influence of natural enemies on brood production in *Ips typographus* (Col. Scolytidae) with special reference to egg-laying and predation by *Thanasimus formicarius* (Col.: Cleridae). *Entomophaga* 37: 333-342.
- WESLIEN J., SCHROEDER M.L. 1999. Population levels of bark beetles and associated insects in managed and unmanaged spruce stands. *Forest Ecology and Management* 115: 267-275.
- WRAIGHT S.P., INGLIS G.D., GOETTEL M.S. 2007. Overview of pathogen groups: Fungi, pp. 223-248. In: Lacey L.A., Kaya H.K. (eds) *Field manual of techniques in invertebrate pathology*. Springer, Dordrecht.
- YAMAN M., BAKI H. 2011. First record of entomopoxvirus of *Ips typographus* (Linnaeus) (Coleoptera: Curculionidae, Scolytidae) for Turkey. *Acta Zoologica Bulgarica* 63: 199-202.
- ZHAO Z.Q. 2006. *Occurrence, taxonomy, biology and pathogenicity of aphelenchid nematodes associated with conifers in south-eastern Australia*. Ph.D. Thesis. Faculty of Sciences, University of Adelaide, Australia, 209 pp.
- ZIMMERMANN G. 2007a. Review on safety of the entomopathogenic fungi *Beauveria bassiana* and *Beauveria brongniartii*. *Biocontrol Science and Technology* 17: 553-596.
- ZIMMERMANN G. 2007b. Review on safety of the entomopathogenic fungus *Metarhizium anisopliae*. *Biocontrol Science and Technology* 17: 879-920.
- ZIMMERMANN G. 2008. The entomopathogenic fungi *Isaria farinosa* (formerly *Paecilomyces farinosus*) and the *Isaria fumosorosea* species complex (formerly *Paecilomyces fumosoroseus*): biology, ecology and use in biological control. *Biocontrol Science and Technology* 18: 865-901.