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Význam patogenů v populační dynamice lýkožroutů rodu *Ips* (Coleoptera: Curculionidae: Scolytinae)

Dizertační práce

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Importance of pathogens in the population dynamics of bark beetles of the genus *Ips* (Coleoptera: Curculionidae: Scolytinae)

Ph.D. Thesis

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Abstrakt

Dizertační práce hodnotí význam patogenů v populační dynamice lýkožroutů rodu *Ips* (Coleoptera: Scolytinae) na základě terénních a laboratorních výsledků stejně jako vychází z již publikovaných vědeckých prací. Práce zahrnuje seznámení a shrnutí informací o jednotlivých skupinách patogenních organismů a stručnou charakteristiku bionomie hostitelských druhů.

Během let 2007-2011 byl získán materiál kůrovců z lapáků, lapačů i z napadených stromů pokácených při nahodilé těžbě. V rámci studia patogenů bylo vypitváno a mikroskopicky analyzováno 12789 jedinců lýkožroutů. Byly získány nové poznatky o patogenech druhů *Ips amitinus* (nový druh prvoka pro tohoto hostitele – *Mattesia schwenkei*) a *I. cembrae* (stanoveno poprvé druhové spektrum a infekční hladiny patogenů) a současně zjištěny nové poznatky o jejich bionomii (výskyt, letová aktivita, obsazování kmene) i rozšíření (*I. amitinus*).

Srovnáním spektra a infekčních hladin patogenů mezi *I. amitinus* a *I. typographus*. Bylo potvrzeno jednak chudší druhové spektrum, stejně tak i nižší infekční hladiny nemocí u *I. amitinus*. Byl zjištěn nárůst infekční hladiny prvoka *Gregarina typographi* během sezóny u matečných brouků *Ips typographus* v požercích a zároveň položen první nepřímý důkaz o předávání nemocí u kůrovců. Při sledování osmi generací *I. typographus* nebyl potvrzen vliv mikrosporidie *Chytridiopsis typographi* a střevních hlístic na populační růst *Ips typograhus* během následujících dvou generací, avšak byla potvrzena pozitivní korelace populačního růstu kůrovců s množstvím přítomných endoparazitoidů.

Práce představuje ucelený přehled nemocí a přináší nové poznatky a zjištění v oboru patologie lesních škůdců. Z komplexních výsledků vyplývá, že patogeny lýkožroutů zřejmě dostatečně neregulují populace kůrovce a zkoumané druhy zřejmě nebrání jim v úspěšném rozmnožování.

Klíčová slova: rod *Ips*, mikrosporidie, gregariny, virus, populační hustota, předávání

Abstract

This PhD thesis evaluates the importance of pathogens on the population dynamics of bark beetles of the genus *Ips* (Coleoptera: Scolytinae) on the basis of field and laboratory research similar to previously published scientific work. This work includes a review and summary of information regarding individual groups of pathogenic organisms and a concise bionomic characterization of the host species.

During the period 2007-2011, bark beetles were collected from pheromone traps, trap trees, and infected felled trees. Within this study of pathogens, about 12,789 individual bark beetles were dissected and microscopically analysed. New findings were obtained about pathogens of the species *Ips amitinus* (new protozoan species for this host – *Mattesia schwenkei*) and *I. cembrae* (species spectrum and infection levels of pathogens determined for the first time). New information about their bionomics (occurrence, flight activity, trunk occupation) and distribution (*I. amitinus*) also was discovered.

A comparison of spectrum and infection levels of pathogens was made between *I. amitinus* and *I. typographus*. *I. amitinus* was determined to have a smaller species spectrum and lower infection levels. A rise in infection levels of the protozoan *Gregarina typographi* over the course of a season was observed for mature beetles of *Ips typographus* in the breeding system, and thus the first indirect evidence of pathogen transmission in bark beetles was established. In monitoring eight generations of *I. typographus*, no impact on population growth was confirmed either for microsporidia *Chytridiopsis typographi* or for intestinal parasites during the subsequent two generations. A positive correlation was demonstrated, however, between population growth and the quantity of endoparasitoids present.

This work presents a comprehensive overview of diseases and yields new information and findings in the field of forest pest pathology. The complex results show that bark beetle pathogens clearly do not regulate beetle populations adequately and that the examined species evidently do not prevent beetles from successfully reproducing.

Key words: genus *Ips*, microsporidia, gregarines, virus, population density, transmission

Prohlášení

Prohlašuji, že jsem dizertační práci na téma „Význam patogenů v populační dynamice lýkožroutů rodu *Ips* (Coleoptera: Curculionidae: Scolytinae)“ vypracovala samostatně a že jsem uvedla všechny literární prameny a publikace, ze kterých jsem čerpala.

V Praze dne 15.8.2012 Mgr. Karolina Lukášová

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Obsah

1 Úvod

V současném lesnickém managementu se stavy lýkožroutů monitorují pomocí klasických metod, jako jsou lapáky, lapače či letecké snímkování. Nejúčinnější pro ochranu porostů však zůstává včasná asanace veškerého napadeného a pro kůrovce atraktivního dříví, které jim umožňuje dostatečný prostor pro rozmnožování a vývin nové generace (Wermelinger 2004). Nové metody vedoucí k redukci populace lýkožroutů jsou v ochraně lesa stále žádány. Aplikace přirozených nepřátel a patogenů do oblastí kůrovcových gradací má v ochraně lesa stále velký potenciál.

Biologický boj je jedna ze strategií „Integrated Pest Management“ (IPM). V ochraně lesa je chápána jako úsilí o obranu před škůdci, jehož podstatou je cílevědomé využití živých („užitečných“) organismů či produktů jimi vytvořených pro udržení škod působených člověku nebo jeho majetku (hospodářským rostlinám a zvířatům) živými („škodlivými“) organismy v ekonomicky únosných mezích, resp. pro jejich snížení či eliminování (Čapek 1994). Použití mikroorganismů pro kontrolu hmyzích škůdců bylo navrženo již před mnoha lety průkopníky v oblasti patologie bezobratlých: Agostino Bassi, Louis Pasteur a Elie Metchnikoff (Steinhaus 1956). Lesnická a zemědělská praxe v Austrálii, Novém Zélandu, Kanadě a USA je v současnosti mnohem dále ve výzkumu regulace importovaných a vypouštěných exotických bezobratlých pro biologickou kontrolu. Tyto země se honosí léty zkušeností s realizací programů biologické kontroly vzhledem k přílivu invazivních nepůvodních druhů škůdců (Coulson et al. 2000).

Mikrobiální patogeny hmyzu napadají a rozmnožují se v tělech hostitelů, z nichž se přenášejí na další jedince. Známe jak potencionální patogeny (potřebují další faktory), tak fakultativní patogeny (potřebují hostitele k rozmnožování resp. replikaci). Mohou se přenášet horizontálně (v rámci jedné generace) kontaktem s kontaminovaným materiálem (trus, zbytky uhynulých jedinců nebo kanibalismus) nebo vektory (např. roztoči, parazitoidi, predátoři atd.) a vertikálně (z rodičů na jejich potomky). Patogeny způsobující infekci mohou být jak nebuněčné organismy (viry), tak prokaryotické (bakterie) a eukaryotické organismy (prvoci, houby, řasy atd.). Infekce u hmyzu může být latentní bez vnějších příznaků či chronické, kdy nejsou letální, ale projevují se různými příznaky, a akutní, když zabíjí hostitele. Působením dalších faktorů se z latentních a chronických onemocnění mohou stát onemocnění akutní. Některé patogeny (hlístice) si pomáhají uvolňováním exo- a endotoxinů, otevírají tím hostitele infekci. Houby do hostitele pronikají přes integument (kontaktně) nebo jako většina ostatních perorálně (ústním otvorem). Patogeny se liší ve virulenci, tj. schopnosti patogenů překonat obranné mechanismy hostitele, namnožit se v něm a následně ho usmrtit (Wegensteiner 2004).

Lýkožrouti rodu *Ips* patří mezi významné škůdce jehličnatých porostů v Evropě (Pfeffer & Knížek 1995; CABI/EPPO 1997; Grodzki 1997; Grodzki 2003; Holuša et al. 2003; OEPP/EPPO 2005; Mazur et al. 2006; Wermelinger et al. 2008). Někteří z nich napadají hned několik hostitelských druhů dřevin. To pravděpodobně také vysvětluje, proč některé patogeny mají více hostitelů a obecně je druhové spektrum nemocí u lýkožroutů velmi podobné. Například hromadinka *Gregarina typographi* (Fuchs, 1915) je známa u všech šesti analyzovaných druhů rodu *Ips* (Takov et al. 2007; Yaman 2007; Holuša et al. 2009; Kereselidze et al. 2010; Takov et al. 2010), nejběžnější mikrosporidie *Chytridiopsis typographi* [(Weiser, 1954) Weiser, 1970] pak u pěti druhů (Wegensteiner 2004; Wegensteiner & Weiser 2004; Holuša et al. 2009; Wegensteiner et al. 2010). Na druhou stranu je znám pouze jeden druhově specifický patogen *Larssoniella duplicati* popsán recentně u *Ips duplicatus* (Sahlberg, 1836) (Weiser et al. 2006; Holuša et al. 2007; Holuša et al. 2009).

V současné době známe kolem 20 druhů patogenních organismů (bez hlístic a entomopatogenních hub) u podčeledi Scolytinae (bývalá čeleď kůrovcovití), z nichž je deset popsáno u lýkožrouta smrkového *Ips typographus* (Linné, 1758) (Wegensteiner 2004; Takov et al. 2010). U většiny patogenů jsou jen zřídka k dispozici informace o vlivu na jedince či populace (na vitalitu, plodnost, letové schopnosti, hibernaci atd.), většina experimentů navíc probíhá pouze v laboratorních podmínkách a terénní studie prakticky chybí. V kapitole 4.1 jsou shrnuty všechny důležité současné poznatky o patogenech (viry, prvoci, houby, zelené řasy) a parazitických hlísticích a poskytují ucelený přehled o zjištěných druzích u různých zástupců rodu *Ips*.

Bionomie a ekologie některých lýkožroutů ještě stále nebyly dostatečně prozkoumány. Naproti tomu existuje celá řada prací o *I. typographus* (Lieutier 2002; Grégoire & Evans 2004; Wermelinger 2004). V dizertační práci byly analyzovány některé biologické charakteristicky, druhová spektra a infekční hladiny patogenů dvou druhů lýkožroutů *Ips amitinus* (Eichhoff, 1871) a *Ips cembrae* (Heer, 1836) (kap. 4.2. a 4.3.).

Distribuce lýkožrouta menšího *I. amitinus*, byla analyzována v České republice a Polsku na hlavní hostitelské dřevině - smrku ztepilém (*Picea abies* (L.) Karst.) (Pfeffer & Skuhravý 1995; Grodzki 1997; Grodzki 2004; Mazur et al. 2006). *I. amitinus* byl zjištěn na více než polovině ze zhruba 40 zkoumaných lokalit od nížin přes střední polohy až po lokality v horských oblastech jako jsou např. Tatry (kapitola 4.2). Podobnou distribuci výskytu *I. amitinus* lze očekávat v celé střední Evropě, v porostech s výskytem smrku. *I. amitinus* běžně napadá především střední partie korunové části stromů, což potvrzují také další práce (Zumr 1984; EPPO/CABI 1997). V místech výskytu vysoké populační hustoty *I. typographus* byla populační hustota *I. amitinus* nízká a obráceně. Naopak předpokládáme obecně velmi podobný a srovnatelně dlouhý vývoj nové generace u *I. typographus* a *I. amitinus*. Lýkožrout menší má jednu generaci ročně v horských oblastech střední Evropy, ale na lokalitách s nadmořskou výškou menší než 600 m n. m. může mít i dvě generace (Zuber 1992; Jurc & Bojović 2004; Grodzki et al. 2008; Turčáni & Hlásny 2007; Witrylak 2008; kapitola 4.2).

Vzhledem k tomu, že druhy *I. amitinus* a *I. typographus* mají podobnou bionomii a mohou se na hostitelských stromech běžně potkávat (EPPO/CABI 1997; Økland & Skarpaas 2008), předpokládali jsme rovněž, že jejich druhové složení a infekční hladiny patogenů budou velmi podobné. Abychom tuto hypotézu otestovali, byli shromážděni brouci vždy ze tří ležících lapáků na osmi studijních lokalitách, kde byly prokazatelně přítomny oba druhy, a určili jsme přítomnou nákazu patogeny. Srovnání bylo provedeno u 938 matečných brouků *I. amitinus* a 3435 jedinců *I. typographus*.

Celkem bylo zjištěno 5 patogenních organismů, dále střevní hlístice a endoparazitoidi. Zásadním objevem byl prvonález neogregariny *Mattesia schwenkei* (Purrini, 1977) v tukovém tělěse u 9% jedinců *I. amitinus*. Průměrná infekční hladina většiny patogenů (*Chytridiopsis typographi*, *Gregarina typographi*, *M. schwenkei* a parazitoidy) byla statisticky významně vyšší u dospělců *I. typographus* než u *I. amitinus*. *Metschnikowia typographi* (Weiser et al., 2003) byla potvrzena pouze u *I. amitinus*, zatímco mikrosporidie *Nosema typographi* (Weiser, 1955) nalezena pouze u *I. typographus*. Navíc bylo zjištěno zvýšení infekční hladiny gregarinou *G. typographi* u *I. amitinus* během sezóny (kapitola 4.3).

Vzrůstající význam a nebezpečí gradací *I. cembrae* v modřínových (*Larix decidua* Mill.) porostech různého stáří (Krehan & Cech 2004; Krehan & Steyer 2005; Grodzki 2008; Knížek 2008; Grodzki & Kosibowicz 2009) vyžadují upřesnění znalostí o bionomii, etologii, reprodukčním potenciálu, populační dynamice, monitoringu, ochraně a obranných opatření proti tomuto škůdci. V letech 2007-2009 byla hodnocena účinnost feromonových lapačů (feromonový odparník Cembräwit ®) v ohniscích gradace *I. cembrae* v České republice. Zároveň byly od konce září umístěny do fotoeklektorů půlmetrové špalky napadené *I. cembrae* a studovány za laboratorních podmínek v měsíčních intervalech (listopad až leden). Na konci března byly položeny lapáky o ploše 0,5 m^2 určené k zachycení brouků opouštějící hrabanku po přezimování. Jarní letová aktivita začala v květnu a kulminovala v polovině měsíce (Postner 1974; Grodzki & Kosibowicz 2009). Stromové lapáky byly napadeny kontinuálně podél celého profilu kmene. Matečné chodby v horní části kmene byly delší a počet nakladených vajíček nižší než ve spodních partiích. Postupný výlet brouků z napadeného modřínového dříví v laboratorních podmínkách v průběhu zimy potvrdily přítomnost přezimování brouků, larev a kukel dceřiné generace ve stromech. Brouci přezimovali v blízkosti stromů, kde ukončili vývoj. Včasné dokončení vývoje tak zvyšuje podíl přezimujících brouků v hrabance blízko napadených stromů (kapitola 4.4).

Vedle studie bionomie bylo u *I. cembrae* poprvé ve střední Evropě zjišťováno i druhové spektrum a hladiny nákaz patogeny. Brouci byli shromážděni z feromonových lapačů, lapáků a fotoeklektorů s napadeným dřívím (*Larix decidua*) v letech 2007 až 2011 na 10 studijních plochách. Celkově bylo mikroskopicky vyšetřeno 3379 matečných brouků. Byly nalezeny dva druhy mikrosporidií (*Ch. typographi* a *Nosema cf. typographi*) a dva druhy hromadinek (*G. typographi* a *M. schwenke*i).

Infekční hladina *Ch. typographi* u populací *I. cembrae* se pohybovala mezi 2 a 58%. Nákaza *Nosema* sp. byla prokázána pouze u dvou jedinců na dvou studijních lokalitách. *G. typographi* byla zaznamenána pouze v Rakousku a Chorvatsku u 1-2% matečných brouků. *M. schwenkei* byla pozorována pouze v Chorvatsku u 0,6% jedinců *I. cembrae*. Pouze jeden houbový patogen rodu *Fusarium* byl nalezen dvou matečných brouků (0,7%) v roce 2010. Spektrum patogenů zjištěné během naší studie u *I. cembrae* bylo velmi podobné druhovému složení nemocí u *I. typographus*. Nebyl zjištěn žádný nový či druhově specifický patogen (kapitola 4.5).

U většiny nalezených patogenů lýkožroutů se předpokládá horizontální přenos, který vyžaduje přímý kontakt s cystami, sferoidy nebo sporami nemocí a jejich následné pozření. (Händel et al. 2003). Nebyl však doložen žádný důkaz o takovém předávání a většina autorů tuto situaci pouze předpokládá (Wegensteiner et al. 1996; Wegensteiner & Weiser 1996; Händel et al. 2003). K předávání údajně dochází především během zralostního žíru nedospělých brouků, kdy často kvůli nedostatku prostoru protínají staré mateční chodby sousedních požerků (Wegensteiner & Weiser 1996), což potvrzuje fakt, že spory patogenů dokážou poměrně dlouho zůstat v inaktivním stavu až do doby kontaktu s hostitelem (Wegensteiner & Weiser 1996).

V naší studii jsme se zaměřili na změny v infekčních hladinách *G. typographi* u matečných brouků. Nárůst nakažených jedinců gregarinou byl studován v populaci *I. typographus* během rozvoje rodinného požerku. Brouci byli opakovaně shromážděni ze tří sekcí na kmenech lapáků v průběhu let 2009 a 2010 na jedné studijní lokalitě v České republice s vysokou hladinou infekce *G. typographi* (roční průměr hladiny nemoci 15,7% v roce 2009 a 19,8% v roce 2010). Nebyly zjištěny statisticky významné rozdíly mezi nákazou u samců a samic, sekcemi ani lapáky, ale průkazně se lišila nákaza mezi jednotlivými odběry. Během reprodukčního období lýkožroutů se infekční hladina patogenů u matečných brouků téměř zdvojnásobila v roce 2009 (10,7 až 19,8%) a více než ztrojnásobila v roce 2010 (z 9,3 na 31,3%). Předpokládáme, že trvalé zvýšení infekce *G. typographi* během obou studovaných sezón vyplývá z přenosu mezi brouky ve snubních komůrkách, tj. horizontálním přenosem během reprodukce a kladení vajíček samicemi v období května až července (kapitola 4.6). Byl tak doložen první nepřímý důkaz o předávání nákaz v rodinných požercích mezi matečnými brouky.

I přes téměř 20-letý intenzivní výzkum patogenů lýkožroutů rodu *Ips* (Wegensteiner 2004; Takov et al. 2006; Burjanadze & Goginashvili 2009; Unal et al. 2009; Takov et al. 2010; Wegensteiner et al. 2010; Lukášová & Holuša 2011; Michalková et al. 2011; Takov et al. 2011; Holuša et al. 2012) chybí stále řada informací o vlivu přítomnosti patogenů na fertilitu, vitalitu, přezimování či letovou aktivitu infikovaných škůdců. Nejasnosti se objevují u přenosu nemocí mezi jednotlivci i generacemi. Dostatečně nejsou popsány ani rozdíly v infekčních hladinách patogenů při použití různých odchytových metod, vyřešena není ani otázka optimální velikosti vzorku hmyzu pro studium patogenů či sezónní změny v hladinách infekce. Pro každý patogenní organismus stejně jako pro každého hmyzího hostitele je potřeba tyto vlastnosti analyzovat zvlášť, což otevírá nové možnosti studia patogenů lesních škůdců i dalších na ně vázaných organismů. Na druhou stranu jsou patogeny dobře popsány z faunistického hlediska (Wegensteiner 2004; Takov et al. 2010; Takov et al. 2011) a mnohdy je i úspěšně prostudována jejich ultrastruktura (Weiser & Wegensteiner 1994; Weiser et al. 1998; Weiser et al. 2003; Weiser et al. 2006) a životní cyklus (Purrini & Žižka 1983; Holuša et al. 2007; Tonka et al. 2010).

Během 4-leté studie jsme jako první podrobně analyzovali vliv patogenů na populační růst lýkožrouta smrkového. Výzkum byl prováděn na třech lokalitách na čtyřech sekcích připravené série lapáků pro jarní a letní generaci na území Vojenského újezdu Libavá. Statistická analýza pomocí korelací infekčních nákaz a koeficientu populačního růstu lýkožroutů nepotvrdila vliv střevních hlístic (u 15 % studovaných jedinců = nižší než zjistili autoři: Burjanadze & Goginashvili (2009); Kereselidze et al. (2010)) a *Ch. typographi* (infekční hladina mezi 0 do 20,4 %, u letní generace vždy vyšší než u jarní) na populační růst lýkožrouta smrkového. Získaná data korespondují s výsledky studie provedené u *G. typographi*, kde rovněž nebyl zjištěn žádný demografický efekt na kůrovce (Wegensteiner et al. 2010). Naproti tomu mezi koeficientem populačního růstu a infekční hladinou endoparazitoidů v populaci (průměrně 5%) byla zjištěna pozitivní korelace. Tedy s populačním růstem lýkožrouta smrkového roste i počet jedinců napadených endoparazitoidy (kapitola 4.7).

Na základě shrnutí všech nových poznatků získaných během výzkumu v rámci dizertační práce byly zpracovány a publikovány nové výsledky v oblasti patologie hmyzu (lýkožroutů rodu *Ips*), které se alespoň částečně snaží odpovědět na nevyřešené otázky.

2 Cíle

1. Zmapovat druhové složení a specifika patogenů brouků podčeledi Scolytinae se zaměřením na *Ips* spp. (kapitola 4.1).
2. Stanovit abundanci, počet generací a druhové spektrum patogenů lýkožrouta menšího (*Ips amitinus*) v podmínkách České republiky a Polska. Srovnat druhové spektrum na studovaných lokalitách s lýkožroutem smrkovým (*Ips typographus*) (kapitola 4.2 a 4.3).
3. Definovat bionomii a následně dosud nepopsané druhové spektrum patogenů u lýkožrouta modřínového (*Ips cembrae*) (kapitola 4.4 a 4.5).
4. Zjistit možnosti předávání infekce mezi matečnými brouky ve snubních komůrkách (kapitola 4.6).
5. V návaznosti na tato zjištění objasnit vliv patogenních organismů na populační růst lýkožroutů (kapitola 4.7).

3 Metodika

3.1 Studijní lokality

Hlavním úskalím při studiu patogenních organismů u kůrovců je vyhledání lokalit s dostatečně promořenými populacemi, při nízkých populačních hustotách a při intenzivním lesnickém managementu jsou totiž infekční hladiny patogenů velmi nízké a druhové spektrum malé. Prvním krokem při řešení tématu byl tedy výběr vhodných studijních ploch. Během let 2007-2011 byla sledována bionomie a hodnoceny infekční hladiny patogenů lýkožroutů rodu *Ips* na lokalitách po celé České republice a částečně také v sousedních státech ve spolupráci se zahraničními kolegy (Polsko, Rakousko, Chorvatsko).

Hlavní studijní plochy v České republice:

Havířov: Studijní lokalita (GPS 49°49'15"N, 18°22'22"E, 265–295 m n. m.), na které byla sledována abundance *I. amitinus* na připravených lapácích (*Picea abies*) v letech 2005-2007.

Hlubočec (GPS 49°50'30"N, 17°58'11"E, 441 m n. m.), **Stařechovice** (GPS 49°34'33"N, 17°04'34"E, 442 m n. m.) a **Šumbark** (GPS 49°47'60"N, 18°24'41"E, 260 m n. m.): Lokality s instalovanými lapáky (*Larix decidua*) určené na odběr brouků *I. cembrae* určených na pitvy a analýzu druhového spektra patogenů v letech 2008-2010.

Ostravice: Studijní lokalita umístěná do centra rezervace PR Smrk (GPS 49°30'07"N, 18°22'12"E) leží v nadmořské výšce mezi 1,180 a 1,200 m n. m. Jedná se o chráněné území a provádí se zde pouze omezený lesnický management reprezentovaný omezenými kůrovcovými těžbami, dovozem lapáků a odchytem brouků do feromonových lapačů. Byly zde sledovány populační hustoty *I. typographus* a *I. amitinus* na připravených lapácích (*Picea abies*) a předávání patogenů ve snubních komůrkách v letech 2009 a 2010.

Potštát (GPS 49°41'21"N, 17°37'11"E, 610 m n. m.); **Kozlov** (GPS 49°37'58"N, 17°30'16"E, 660 m n. m.) a **Staré Odřůvky** (GPS 49°43'01"N, 17°36'32"E, 500 m n. m.): Po dobu 8 po sobě jdoucích generacích (od 2008 do 2011) jsme sledovali vývoj nákaz *I. typographus* na třech lokalitách v prostoru Vojenského újezdu Libavá. Materiál matečných brouků byl shromážděn z 5 až 10 stromů (přilehlé stromy byly asi 100 m od sebe) na každé lokalitě.

Pustá Polom (GPS 49°51'01"N, 17°59'50"E, 434 m n. m.): V roce 2010 byly odebrány vzorky z ležících lapáků (*L. decidua*) na zjištění patogenů *I. cembrae*.

Slezské Rudoltice (GPS 50°12'37"N, 17°38'52"E, 275 m n. m.): V letech 2007 a 2008 zde byly umístěny lapáky (*L. decidua*) i lapače (feromonový odparník Cembräwit®) na monitoring bionomie *Ips cembrae*. V získaném materiálu jsme analyzovali také složení patogenů.

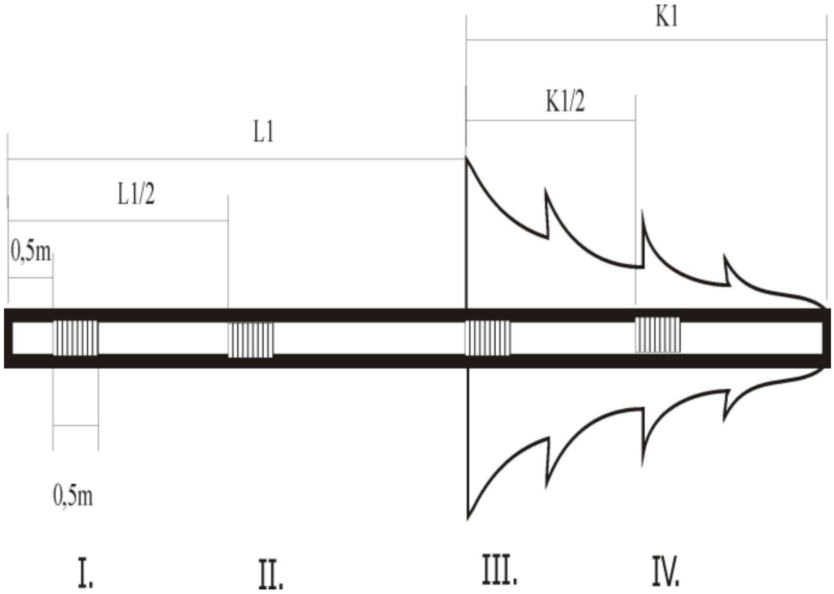
Útěchov (GPS 49°17'12"N, 16°37'15"E, 450 m n. m.): V letech 2008 a 2009 zde byly umístěny lapáky (*L. decidua*) i lapače (feromonový odparník Cembräwit®) na monitoring bionomie a populační hustoty *Ips cembrae*. Z tří lapáků v roce 2009 byly odebrány a analyzovány vzorky brouků na určení patogenů a jejich infekčních hladin.

3.2 Sběr a zpracování vzorků

Čas odběru byl určen na základě zahájení nové generace brouků – tyto termíny byly svázány s chodem teploty a srážek během vegetačního období. Na jaře (červen) byli zjišťováni dospělci rodičovské generace (P). V létě proběhla druhá perioda vzorkování (srpen), kde byli nasbíráni brouci dceřiné generace (F1) ze snubních komůrek a matečných chodeb, protože patogeny se snadněji zjišťují u dospělých brouků (Wegensteiner & Weiser 1996a). Mateční brouci byli sbíráni individuálně z lapáků a napadených stromů nebo hromadně sesypáním z lapačů do plastových lahví. Pro každou lokalitu a období odběru vzorků bylo shromážděno vždy maximum brouků z každé půlmetrové sekce stromu. Nejčastěji byli odebírání po rodinách z půl až metrových sekcích stromu (nejčastěji 4 sekce: I. 0,5m od paty kmene, II. v polovině kmene, III. začátek zeleného větvení, IV. polovina koruny; Obr. 1).

Získaní brouci byli spolu s kouskem navlhčené gázy nebo vaty (= udržení vlhkosti ve vzorcích) uloženi v plastových nádobách typu 2-cm^3 Eppendorf do chladu při -5^{o}C. Postupně byli brouci pitváni (do 3 měsíců od odběru) pomocí chirurgických pinzet do kapky vody. Vzorky byly sledovány pod světelným mikroskopem Arsenal LPE 5013i-T pod zvětšením 100x – 400x. Inspekce se zaměřila především na trávicí soustavu, Malphigické trubice, gonády a tukové těleso, kde jsou patogeny nejčastěji lokalizovány. Z každé pitvy byl veden detailní záznam o stavu a početnosti infekce a vnitřních orgánů do připraveného formuláře.

Pozornost byla věnována především mikrosporidiím, prvokům, virům, endoparazitoidům a hlísticím. Pozitivní nálezy byly vyfotografovány a změřeny rozměry cyst nebo spor patogenů (program Atlas 3.5.12.0).



Obr. 1 Rozložení sekcí a jejich vzdálenosti od paty stromu.

Populační hustota lýkožroutů na jednotlivých sekcích lapáků byla zjišťována při studiu populací na všech lokalitách během celého výzkumu. Na každé odkorněné sekci byl kalkulován počet rodin (= počet snubních komůrek, stádium vývoje, délka chodeb, počet nakladených vajec a míra parazitace) lýkožrouta smrkového a přepočtena jejich hustota na jednotku plochy podle velikosti studované sekce (délka přibližně 0,5 m a šířka cca polovina obvodu kmene). Data byla zapsána a poté převedena do tabulkového softwaru pro další analýzu.

Pro výpočet korelace mezi infekční hladinou patogenů a nárůstem populační hustoty byl použit koeficient populačního růstu z roku na rok podle vzorce pro výpočet rychlosti růstu:

$$R = \log N_t - \log N_{t-1}$$ (Jarošík 2005).

3.3 Analýza dat

Získaná data byla převedena do tabulkového softwaru MS Excel 2007 a poté graficky a statisticky vyhodnoceny v programu Statistica 9. Při analýzách byla vždy sledována normalita dat (Shapiro test) a následně provedeny další pokročilé analýzy zjišťující rozdíly v infekčních hladinách patogenů v jednotlivých datech, na různých lokalitách, v pohlaví či u různých druhů (ANOVA, Kruskal Wallis test, t-test, Wilcox test atd.) nebo korelace mezi infekční hladinou patogenů a populačním růstem lýkožroutů rodu *Ips*. Při vyhotovení map výskytu jednotlivých patogenů a druhů kůrovců byl použit program ArcMap 10 v prostředí GIS. Obrázky a fotografie byly graficky upravovány v programu AdobePhotoshop 7.0 CE.

4 Výsledky

Předkládaná dizertační práce je postavena na 7 článcích, jež jsou citovány v textu a uvedeny v kapitolách 4.1-4.7. Dva z článku již byly publikovány v časopisech Acta Protozoologica a Journal of Applied Entomology. Tři články byly přijaty k publikaci v časopisech: Zprávy lesnického výzkumu, Acta Zoologica Bulgarica a Journal of Applied Entomology. Jeden manuskript je v současnosti v recenzním řízení v časopise Šumarski list a poslední je rukopis před dopracováním. Hlavními výsledky výzkumu v rámci dizertační práce jsou články v časopisech s IF nebo v databázi Scopus a jeden rukopis:

4.1 Patogeny lýkožroutů rodu *Ips* (Coleoptera: Curculionidae: Scolytinae): review

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2012

Zprávy lesnického výzkumu, přijato k publikování.

PATOGENY LÝKOŽROUTŮ RODU *IPS* (COLEOPTERA: CURCULIONIDAE: SCOLYTINAE): REVIEW

PATHOGENS OF BARK BEETLES OF THE GENUS *IPS* (COLEOPTERA: CURCULIONIDAE: SCOLYTINAE): REVIEW

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ABSTRACT

Some bark beetles of genus *Ips* occuring in Europe attack multiple tree hosts. This probably explains why some of the bark beetle diseases have also more hosts. For example *Gregarina typographi* was known from all of six analysed species and *Chytridiopsis typographi* from midgut epithelium of five species of the genus *Ips*. On the other hand there is only one known species-specific pathogen *Larssoniella typographi* described in *Ips duplicatus*. At present, around twenty species of pathogens were described in bark beetle subfamily Scolytinae, out of which ten are known in the spruce bark beetle *Ips typographus*. For most pathogens, only rare information is available about influence on beetles: on the vitality, fertility, flight ability, hibernation, etc. Moreover, most experiments are conducted under laboratory conditions. In this paper we summarize the knowledge of pathogens (viruses, protozoa, fungi) and parasitic nematodes and give a comprehensive overview of detected pathogens in different species of genus *Ips*.

Klíčová slova: rod *Ips*, mikrosporidie, gregariny, virus, populační hustota, předávání

Key words: genus *Ips*, microsporidia, gregarines, virus, population density, transmission

Úvod

Lýkožrouti rodu *Ips* (Coleoptera: Curculionidae: Scolytinae) napadají ve střední Evropě jehličnaté porosty především smrku ztepilého (*Picea abies* (L.) Karst.), např. *Ips typographus* (L., 1758), *I. amitinus* (Eichhoff, 1871) (GRODZKI 1997; CABI/EPPO 1997; MAZUR et al. 2006) a *I. duplicatus* (Sahlberg, 1836) (PFEFFER, KNÍŽEK 1995; CABI/EPPO 1997; GRODZKI 2003; HOLUŠA et al. 2003). Borovice *Pinus sylvestris* L. a další zástupce rodu *Pinus* preferuje *Ips acuminatus* (Gyllenhal, 1827) a *I. sexdentatus* (Börner, 1776) (BAKKE 1968; CABI/EPPO 1997; WERMELINGER et al. 2008). Na modřín *Larix decidua* Miller je jako na hlavní hostitelskou rostlinu vázán *Ips cembrae* (Heer, 1836) (POSTNER 1974; OEPP/EPPO 2005). Hostitelské druhy dřevin se mohou příležitostně překrývat a jednotlivé druhy lýkožroutů se vzhledem ke své podobné bionomii potkávají a nejspíše si i předávájí patogenní organismy.

V současnosti známe několik desítek druhů patogenů (tab. 1) a hlístic, které byly nalezeny v tělech lýkožroutů rodu *Ips*. První popisy pocházejí z počátku 20. století (FUCHS 1915), další až z poloviny 20. století. Intenzivně jsou nemoci studovány od poloviny devadesátých let (WEGENSTEINER 2004). V té době bylo objeveno nejvíce druhů, poslední pak v roce 2006 (WEISER et al. 2006). Přehled všech nemocí (mikrosporidie a prvoci) byl zpracován TAKOVEM et al. (2010). Ve většině případů známe jen ultrastrukturu nemocí a výše infekčních nákaz různých populací, avšak bez vztahu k početnosti hostitele. U většiny druhů neznáme vývojové cykly nebo v současné době probíhá jejich výzkum. Ještě slabší jsou znalosti o hlísticích. Všechny druhy vázané na rod *Ips* byly zpracovány v monografii RÜHMA (1956), od té doby bylo napsáno jen několik studií na toto téma.

V této práci si klademe za cíl kompletně shrnout znalosti o patogenech a hlísticích parazitujících v lýkožroutech rodu *Ips*. Poslední podobné shrnutí se objevilo v práci WEISERA (2002) o lýkožroutu smrkovém a WEGENSTEINERA (2004).

Viry

Virová onemocnění jsou druhově specifická, selektivní a způsobují rozpad hostitelských tkání v kalnou tekutinu. Mléčné zbarvení způsobují bílkovinné polyedry, které se před úhynem jedince objevují v tukovém tělese. Nejčastějšími hostiteli jsou housenky motýlů. Vnějším příznakem patogenu je masové hynutí larválních instarů na vrcholcích větví či listů (WEISER 1966). Kultivace virů je reálná pouze za laboratorních podmínek, protože vyžaduje přesné technologické postupy.

Entomopoxvirus známý u *Ips typographus* (*ItEPV*, obr. 1) a dalších druhů rodu *Ips* se projevuje tvorbou bílkovinných světlolomných inkluzí pouze ve stěně střeva dospělých brouků, jiné orgány nenapadá (WEISER, WEGENSTEINER 1994; WEGENSTEINER, WEISER 1995; WEISER et al. 2000; BURJANADZE, GOGINASHVILI 2009; YAMAN, BAKI 2011). Čočkovité inkluze obsahují větší množství sendvičovitých virových částic (hranaté), postupně vyplňují střevní epitel a uvolňují se s výkaly (WEGENSTEINER 2004). Může dojít až k perforaci střeva a úmrtí hostitele. V České republice je tento virus zaznamenán především z Šumavy (WEISER et al. 2000; WEISER 2002). Patogen se přenáší trusem a předává se během úživného žíru. Aplikace infekce byla dosažena ošetřenými poleny lapáku (rozdrcení nakažení brouci a feromonový odparník) (PULTAR, WEISER 2004). Další práce studující efektivitu těchto přenosů a vlivů na brouky nepřinesly uspokojivé výsledky (HOLUŠA et al. 2004). HÄNDEL et al. (2001) nalezl *ItEPV* také u *I. amitinus* v Rakousku.

Svými vlastnostmi (selektivita, účinnost) patří viry mezi potencionálně nejvhodnější prostředky biologického boje s lesními i zemědělskými škůdci. Výsledky terénních pokusů však momentálně nejsou uspokojivé a předběžné výsledky naznačují, že viry v boji proti lýkožroutu smrkovému nebudou efektivní. Jejich aplikace naráží na obtížnou izolaci a komplikace při výrobě.

Prvoci (Protozoa)

Mezi patogeny lesních a zemědělských škůdců řadíme také prvoky, především měňavky, hromadinky a kokcidie (WEISER 1966). Jejich význam v použití v biologickém boji proti škůdcům je omezený, některé druhy např. gregariny jsou někdy označovány spíše jako komenzální organismy a neogregariny napadající tukové těleso se velice špatně šíří (prakticky dojde k předání nemoci až po uhynutí a rozkladu hostitelských tkání). S druhem *Malamoeba scolyti* (Purrini, 1980) a *Menzbieria chalcographi* (Weiser, 1955) byly provedeny jedny z prvních infekčních pokusů u kůrovce *Pityogenes chalcographus* (L., 1761) (PURRINI, FÜHRER 1979). Kokcidie se u kůrovců nevyskytují.

- **Měňavky (Rhizopoda)**

V Malphigických trubicích a střevě hmyzu se usazují měňavky rodu *Malamoeba*, u kůrovců se jedná o druh *Malamoeba scolyti* (Rhizopoda, Amoebidae) popsaný poprvé u druhu *Dryocoetes autographus* (Ratzeburg, 1837). Potvrzen byl u dvou druhů lýkožroutů: ve střevě *I. typographus* (WEGENSTEINER 1994; WEGENSTEINER et al. 1996; HÄNDEL et al. 2001) a *I. acuminatus* (ZITTERER 2002). Celý životní cyklus *M. scolyti* u *D. autographus* popsali PURRINI, ŽIŽKA (1983). Tento patogen se vyznačuje se velkými vejčitými cystami, které z těla hostitele odcházejí přes zadní střevo spolu s trusem. Pokud se namnoží, může dojít k ucpání trubic, čímž je znemožněno vyměšování odpadních látek z těla (WEISER 2002). Byla provedena experimentální infekce dalších kůrovců včetně zástupců rodu *Ips* (*I. typographus, I. acuminatus, I. sexdentatus*) a na rozdíl od kontrolních jedinců došlo ke zkrácení délky života brouků zhruba na polovinu (KIRCHHOFF, FÜHRER 1990).

- **Hromadinky (Apicomplexa)**

Hromadinky jsou obligátní paraziti bezobratlých zahrnující tradičně tři skupiny: Archigregarinida, Eugregarinida a Neogregarinida (THÉODORIDÉS 1984). V posledních letech byla prokázána jejich fylogenetická příbuznost se zástupci rodu *Cryptosporidium*, významnou skupinou patogenů obratlovců (BARTA, THOMPSON 2006). Obecně jsou nové druhy řádu eugregarin popisovány na základě kritérií jako je obecná morfologie, morfometrie, tvar epimeritu, velikost a tvar a načasování spojení gamontů (SMITH, COOK 2008). Dnes je popsáno více než 1 700 druhů gregarin z přibližně 3 200 hostitelských druhů (CLOPTON 2000).

Zástupci těchto parazitů se běžně vyskytují u hmyzu (ZUK 1987; SIMMONS 1990; LANGE, WITTENSTEIN 2002; SMITH et al. 2007; SMITH, COOK 2008; LOCKLIN, VODOPICH 2010), zvláště u brouků (Coleoptera) (SCHAWANG, JANOVY 2001; YAMAN 2002; YAMAN 2007; SIENKIEWICZ, LIPA 2008). Z hmyzích druhů bylo pouze 0,32 % známých taxonů studováno na přítomnost eugregarin (CLOPTON 2000).

Eugregarina *Gregarina typographi* (Fuchs, 1915) byla zjištěna ve střední části střeva u celé řady zástupců podčeledi Scolytinae (TAKOV et al. 2007; YAMAN 2007; HOLUŠA et al. 2009; KERESELIDZE et al. 2010; TAKOV et al. 2010). Gregariny se vyznačují několika vzhledově odlišnými stadii. Mají přímý vývojový cyklus - nemají žádného mezihostitele nebo vektor přenosu (CLOPTON, GOLD 1996). Kůrovci se infikují pozřením oocyst v nakaženém trusu, zbytcích těla uhynulých jedinců a kanibalismem při tvorbě požerku nebo při zralostním žíru. Po pozření oocyst je aktivována excystace ve střevním epitelu, uvolnění sporozoiti prodělávají v trávicí soustavě vnitrobuněčný vývoj mezi mikrovily střevního epitelu (TRONCHIN, SCHRÉVEL 1977) a dorůstají do tzv. trofozoitů. Trofozoiti zůstávají spojeni se střevním epitelem pomocí epimeritu (OMOTO et al. 2004; SMITH et al. 2007; VALIGUROVÁ et al. 2009) a prodělávají extracelulární růst. V pohlavní fázi se vždy dva trofozoiti spojují do dvojic jako haploidní gamonti – tento proces se nazývá syzygie a je zakončen vznikem reproduktivní gametocysty (obr. 2 – 4). Gametocysta vychází z těla hostitele spolu s trusem.

Ve vnějším prostředí probíhá mixie a sporogonie. Gametocysty praskají po uzrání a životní cyklus začíná znovu (CLOPTON, GOLD 1996; OMOTO et al. 2004; TOSO, OMOTO 2007).

Ze současných výzkumů je jasné, že *G. typographi* není pravděpodobně velmi virulentní patogen (YAMAN 2007; WEGENSTEINER et al. 2010). Obecně gregariny zřejmě způsobují mechanická a fyziologická poškození střevního epitelu, ovlivňují vylučování metabolitů a toxinů během procesu pinocytózy. Vývoj trofozoitů poškozuje buňky ve střevním epitelu a poskytuje tak vstupní bránu do tělní dutiny pro další patogeny (LIPA 1967). Poškození středního střeva však mohou být lehce kompenzována buněčnou regenerací a patologický efekt na hostitele je obecně velmi slabý (LIPA 1967; TANADA, KAYA 1993). V případě vysokého počtu trofozoitů může jedinec zemřít na ucpání střeva (CERYNGIER, HODEK 1996).

Tukové těleso hostitelských druhů hmyzu je napadáno a ničeno schizogregarinami (Apicomplexa, Neogregarinida). Onemocnění způsobuje během merogonie a sporogonie patogena lýzi buněk tukového tělesa (PERKINS 2000), čímž zřejmě zvyšuje úmrtnost přezimujících jedinců. *Menzbieria chalcographi* sporuluje v kulovitých cystách s 30 sporami. Cysty se v nakaženém jedinci rozpadají na protáhle vejčité až člunkovité spory, jejichž počet dosahuje až 100 tisíc. Uvolní se až po uhynutí hostitele. Nakažení jedinci většinou nevylétají a zůstávají v chodbičkách po úživném žíru a k přenosu tak dochází až po rozložení jedince (WEISER et al. 2000). Obecně se jedná o poměrně vzácný patogen rodu *Ips* (WEGENSTEINER, WEISER 2004; HOLUŠA et al. 2009). Nákazu je možné uměle rozšířit postřikem suspenze nakažených jedinců na povrch lapáku (HOLUŠA, WEISER 2005). Původně popsaný další druh neogregariny *Telosporidium typographi* (FUCHS 1915) z tukové tkáně *I. typographus* byl podle morfologické charakteristiky nejspíše zástupce rodu *Mattesia* (obr. 1), popsaného později dalšími autory (WEGENSTEINER, WEISER 1996a; HÄNDEL et al. 2003). U dalších druhů škůdců jsou neogregariny zaznamenány zřídka (WEGENSTEINER 2004).

Houby (Fungi)

Řada houbových onemocnění je spíše sekundárním faktorem objevující se až po úhynu jedinců způsobeným jinými faktory jako poškození a přehřátí.

Mezi hlavní houbové patogeny škůdců patří především *Beauveria bassiana* (Bals.) a *Beauveria brongniartii* (Sac.) pokrývající tělo hostitelů hustým bílým povlakem mycelií a konidiemi („bílé muskardiny“). *B. bassiana* je popsána jako patogen vyskytující se u více než

100 druhů hmyzu (HAJEK, ST. LEGER 1994). Tato houba je potencionálním nástrojem pro biologickou kontrolu mnoha hospodářských škůdců a hodnocena jako náhrada za běžně užívané chemické pesticidy (ROBERTS, HAJEK 1992). Průkazně hojněji se objevuje v lesních biotopech, zatímco další entomopatogenní houba *Metarhizium anisopliae* (Metch.) více v zemědělských biotopech (VANNINEN 1996; BIDOCHKA et al. 2002).

V ČR byl registrován biopreparát Boverol obsahující prášek s konidiemi entomopatogenní houby *B. bassiana*. Preparát byl primárně určen proti mandelince bramborové, ale je účinný i proti dalším škůdcům, např. housenicím pilatek, obalečům, ponravám chroustů (školky i koruny stromů) a larvám v půdě (klikoroh, lalokonosci, ponravy) (WEISER 1966). Využívání biopreparátů na bázi *B. bassiana* proti *I. typographus* je rozšířeno zejména v Německu, Švýcarsku a Rakousku, na experimentální úrovni je tato houba zkoušena i v dalších zemích (např. USA, Austrálie, Finsko, Polsko). Nejčastěji je *B. bassiana* aplikována formou vodních suspenzí spor na povrch napadených stromů nebo stromových lapáků.

U brouků získaných na Šumavě z feromonových lapačů je nejběžnější entomogenní houbou *Verticillium lecanii* (Zimm.) Viegas, zatímco u brouků na stromech (*P. abies*) je nejběžnější *B. bassiana* (LANDA et al. 2001). Výsledky laboratorních studií dokazují, že v porovnání s většinou ostatních druhů entomopatogenních hub vykazuje *B. bassiana* po aplikaci na dospělce *I. typographus* nejen nejvyšší virulenci (WEGENSTEINER 1996; KREUTZ et al. 2004) – hlavně izoláty získané z čistých kultur z mrtvých hostitelů (DRAGANOVA et al. 2007), ale i zjevně největší adaptaci na tohoto hostitele ve smyslu autodiseminace (samošíření), tj. schopnosti patogenů šířit se prostřednictvím přirozených mechanismů odrážejících populační chování hostitele (LANDA et al. 2007).

Příkladem praktického využívání tohoto jevu je i unikátní forma aplikace *B. bassiana*, při které je práškový koncentrát konidií patogena aplikován přímo do sběrné části feromonového lapače, který je upraven tak, aby byla zachována jeho atraktantní funkce, nicméně dospělci kůrovce nejsou natrvalo odchyceni sběrnou částí lapače. Cílem této aplikace je kontaminovat povrch těla dospělců vysokou dávkou spor a zajistit tak nejen usmrcení konkrétního jedince kontaminovaného při průchodu lapačem, ale zároveň podpořit i šíření nákazy v lokální populaci. Praktické výsledky prokázaly, že tato forma využití biopreparátů na bázi *B. bassiana* je velmi účelná zejména v situacích, kdy od aplikace není očekáván okamžitý účinek, ale perspektiva dlouhodobějšího potlačování populací škůdce (LANDA et al. 2007). Předpokládané vysoké účinnosti pravděpodobně nebude dosaženo, protože od těchto pokusů uplynulo již 5 let a nejsou k dispozici uspokojivé výsledky o vlivu na populace kůrovce.

Mezi další houbové entomopatogeny řadíme např. příležitostný patogen v hemolymfě a střevním epitelu kůrovců: kvasinka *Metschnikowia typographi* (Ascomycota: Metschnikowiaceae) (WEISER et al. 2003; UNAL et al. 2009).

Mikrosporidie (Zygomycetes: Microsporidia)

Tato skupina striktně intracelulárních parazitů byla dříve řazena k prvokům, dnes se považují za primitivní houby (CORRADI, KEELING 2009; REDHEAD et al. 2009). Jedná se přibližně o 1 300 oficiálně popsaných druhů = 160 rodů (WITTNER, WEISS 1999). Mikrosporidie jsou nejčastěji se vyskytující patogeny lesních i zemědělských hmyzích škůdců, vyvíjejí se ve všech tkáních a vývojových stádiích hostitele. K infekci dochází nejčastěji pozřením nakažené potravy (HOLUŠA, WEISER 2005). Pouze v případě velmi silné infekce můžou mikrosporidie napadnout vaječníky a objevuje se transovariální přenos (WEISER et al. 2000; PHELPS, GOODWIN 2008).

Mikrosporidie mají uniformní životní cyklus (CALI, TAKVORIAN 1999). Zralá spóra obsahuje typický vystřelovací aparát. V klidovém stavu je jeho hlavní složkou spirálně svinutá pólová trubice, posteriorní vakuola a polaroblast (= systém membrán ohraničený dutinami v přední části spory, většinou objemná struktura obklopující rovnou část pólového vlákna a končící na úrovni prvního závitu vlákna) (FRANZEN 2004, 2005; XU, WEISS 2005; DELBAC, POLONAIS 2008).

Klíčící spora vstřikuje svůj obsah ve formě malé buňky = sporoplazmy do cytoplasmy hostitelské buňky. Sporoplazma roste a dělí se pomocí merogonie do buněk zvaných meronti, které se dál dělí a postupně vyplňují cytoplasmu hostitelské buňky. Pak je na základě neznámého signálu aktivována syntéza bílkovin, které tvoří stěny spory, buněčnou stěnu obsahující chitin a specifické proteiny mikrosporidií (BOHNE et al. 2000; HAYMAN et al. 2001; BROSSON et al. 2005; PEUVEL-FANGET et al. 2006), které jsou postupně ukládány na plazmatické membráně stádia sporontů. Sporonti se mohou ještě na chvíli rozdělit v procesu sporogonie, ale nakonec každá sporontní buňka dává vzniknout komplexnímu složení spory včetně vystřelovacího aparátu. Spora, která je výsledkem vnitřní diferenciace jediné buňky (VÁVRA, LARSSON 1999), je jediná fáze objevující se volně a je to stádium zodpovědné za šíření patogenu.

U kůrovců je známo několik druhů mikrosporidií. Tyto druhy napadají střevní epitel středního střeva, dostávají se do vaječníků a jsou předávány larvám (WEISER et al. 1998; WEISER 2002;

WEGENSTEINER 2004). U larev se výskyt onemocnění a mortalita neprojevuje. Hodnoty infekční hladiny nad 30 % u viru a mikrosporídií považuje bez hlubšího studia WEISER (2002) za příznak nastupujícího zániku ohniska přemnožení brouků pomocí přirozené regulace populace.

Unikaryon montanum (Weiser, Wegensteiner, Žižka, 1998) je nalézán v tukové tkáni, Malphigických trubicích a vaječnících lýkožrouta smrkového (WEGENSTEINER, WEISER 2004). *Nosema typographi* (Weiser, 1955) je lokalizován v tukové tkáni, ováriích a Malphigických trubicích. Má dvoujaderné spory odcházející s trusem (PURRINI 1978; WEGENSTEINER, WEISER 1996b). Promoření v populacích *I. typographus* je běžně velmi nízké, pohybuje se kolem 2 % i méně (WEGENSTEINER, WEISER 1996b; HÄNDEL et al. 2003).

Nejběžnější mikrosporidie *Chytridiopsis typographi* (původně *Haplosporidium typographi*) [(Weiser, 1954) Weiser, 1970] (obr. 5) se vyznačuje tvorbou velmi odolných silnostěnných cyst se 16 – 32 kulovitými sporami, které jsou infekční agens, a absencí polaroblastu nahrazeného polárními vlákny v trvalém polárním vaku (WEGENSTEINER 2004; TAKOV et al. 2010, 2011; TONKA et al. 2010; WEGENSTEINER et al. 2010; MICHALKOVÁ et al. 2011). *Ch. typographi* vytváří vředovitá ohniska, kde dochází k porušení střeva. Vyznačuje se dvěma typy spor: tenkostěnnými – na vnější prostředí méně odolnými a silnostěnnými. Tenkostěnné spory, produkované v tenkostěnných dočasných sporoforních váčcích, šíří infekci uvnitř hostitele tím, že vstříknou sporoplazmu do epitelových buněk střeva původního hostitele. Silnostěnné spory jsou umístěny v trvalém sporoforním váčku připomínajícím cystu, který neuvolňuje spory v původním hostiteli, ale je vylučován s trusem. Tyto spory zůstávají infekční po dobu několika měsíců ve vnějším prostředí a slouží pro horizontální přenos patogena. Kromě toho má *Ch. typographi* rané vývojové stádium s vícejadernými mateřskými buňkami, z nichž každá vytváří jeden pupen v epitelu středního střeva. Kulovitý pupen je spojen s mateřskou buňkou límcem a buněčné složky jsou tlačeny ke vzdálenému konci pupenu. Obě mateřské buňky a pupen pokračují ve vývoji, pupen se poté oddělí od mateřské buňky, roste a produkuje buňky stejného typu. Obě buňky pak pokračují ve sporogoniálním vývoji a vytváří cysty s výtrusy. Proces, kdy jedna mateřská buňka produkuje jediný pupen rostoucí do stejného strukturovaného a velkého stádia, nemá obdobu u jiných mikrosporidií (TONKA et al. 2010). Obecně je *Ch. typographi* nespecifický patogen napadající pouze epitel střeva řady zástupců podčeledi Scolytinae (WEGENSTEINER 2004). Infekční hladina tohoto patogenu variuje většinou v řádech desítek procent (WEGENSTEINER 2004; WEGENSTEINER, WEISER 2004; HOLUŠA et al. 2009; WEGENSTEINER et al. 2010).

Nedávno byla popsána druhově specifická mikrosporidie vázaná na *I. duplicatus*, *Larssoniella duplicati* (Weiser, Holuša, Žižka, 2006) (HOLUŠA et al. 2007; obr. 6) z východu České republiky a severovýchodního Polska. Jde o chronickou, široce rozšířenou nákazu. Infekční hladina tohoto patogenu dosahuje na některých lokalitách až 80 % (HOLUŠA et al. 2009). Má drobné jednojaderné spory, nákaza probíhá v podélných a okružních svalech středního střeva a v matrix tracheálních zakončení na povrchu střeva (WEISER et al. 2006; HOLUŠA et al. 2007; HOLUŠA et al. 2009). Infekční hladina *L. duplicati* se nelišila mezi generacemi lýkožroutů a byla relativně stálá i u přezimujících jedinců, z čehož vyplývá, že docházelo k horizontálnímu přenosu skrz požití spor (HOLUŠA et al. 2009). U lýkožroutů rodu *Ips* se dá předpokládat výskyt dalších mikrosporidií, např. *Canningia spinidentis* (Weiser, Wegensteiner, Žižka, 1995) popsané z tukové tkáně, Malphigických trubic, svalů a pojivové tkáně rodů *Pityokteines* a *Tomicus* (WEISER et al. 1995).

Mikrosporidie vyvolávají společné epizootie s virem polyedrie u gradujících škůdců, např. bekyně a roztékající se virotická housenka rovněž uvolňuje spory mikrosporidie, které by jinak čekaly na uhynutí hostitele déle. Mikrosporidie samotné vytvářejí chronická onemocnění, se zřetelnou mortalitou u housenek bekyní nebo housenic pilatek hlavně v kombinaci s virózou (HOLUŠA, WEISER 2005). Tyto patogeny jsou skupinově specifické pro určité hostitele, jejich namnožování je možné v laboratorních chovech, ale přípravek na bázi mikrosporidií proti kůrovcům není k dispozici na trhu insekticidů (WEGENSTEINER 2004). Hlavním důvodem je obtížná masová kultivace s nutností namnožení v živých hostitelích, které je obtížně praktikovatelné vzhledem ke kryptickému způsobu života lýkožroutů rodu *Ips* a jejich izolovanému vývinu jednotlivých stadií v prostoru.

Hlístice (Nematoda)

Entomopatogenní hlístice (Nematoda: Heterorhabditidae, Mermithidae, Steinernematidae) jsou letální endoparazité hmyzu (GAUGLER, KAYA 1990; GAUGLER 2002). Jsou běžně užívány v biologickém boji proti druhům hmyzu žijícím kryptickým způsobem života (RAMOS-RODRÍGUEZ et al. 2006), nejběžněji ve vlhkém a v půdním prostředí (KAYA, GAUGLER 1993). Jejich výhodou je nízká patogenita pro obratlovce (KAYA, GAUGLER 1993; BATHON 1996).

Do tělní dutiny hostitelů se hlístice dostávají tělními otvory (stigmata, ústní otvor nebo kutikula – rod *Heterorhabditis*). Po vniknutí do hostitele vypouštějí pomocí symbiotických bakterií rodů *Xenorhabdus* a *Photorhabdus* (FORST et al. 1997) endotoxiny, kterými svého hostitele zabíjí. Po usmrcení hostitele slouží jeho tělo dále spolu s bakteriemi jako živná půda pro vývoj hlístic. Infekční juvenilní stádia (desítky až stovky tisíc) opouští usmrceného hostitele přibližně po 2 – 3 týdnech a aktivně (pachově pomocí hlavových papil a amphid) vyhledávají nové živé jedince hmyzu ke kolonizaci (CROLL 1970).

Hlístice jsou testovány jako efektivní proti široké škále hmyzu v různých prostředích proti škůdcům jako jsou švábi (KOEHLER et al. 1992), obaleči, bekyně (WEISER, MRÁČEK 1988) a další z čeledí Pyralidae (SHANNAG, CAPINERA 2000) a Curculionidae (DUNCAN, MCCOY 1996; SHAPIRO, MCCOY 2000). Někteří autoři udávají souvislost výskytu hlístic s gradacemi některých škůdců (MRÁČEK, BEČVÁŘ 2000) a jejich regulací, např. ploskohřbetky: rod *Cephalcia* (odhady kolem 30 %) (MRÁČEK 1986). Hlístice nepatří k běžným patogenům kůrovců, ale mohou je úspěšně infikovat (POINAR, DESCHAMPS 1981); experimentální aplikace hlístic se však momentálně neprovádějí.

Hlístice využívají kůrovce k přesunu na nová stanoviště (forézie) nebo je potřebují k dokončení svého vývojového cyklu (parazitace). Mezi hlístice s vazbou ke kůrovcům řadíme především zástupce řádů Tylenchida a Rhabditida. Většina hlístic asociovaných s lýkožroutem smrkovým brouky negativně neovlivňuje, ale existují i některé parazitické druhy (RÜHM 1956). Ty jsou lokalizovány v těle brouků buď volně v hemolymfě: rody *Contortylenchus* a *Parasitylenchus*, nebo v Malphigických trubicích: rod *Cryptaphelenchus,* či ve střevě: rody *Aphelenchoides* a *Parasitorhabditis* (RÜHM 1956). Průměrná nákaza kůrovců střevními hlísticemi se pohybuje kolem 50 % (WEGENSTEINER, WEISER 1996b; BURJANADZE, GOGINASHVILI 2009; KERESELIDZE et al. 2010). Podle některých studií zabíjejí parazitické hlístice své hostitele (způsobují ucpání střeva a jeho perforaci) a redukují jejich životnost a plodnost (LIEUTIER 1980; KAYA 1984), např. zmenšením oocytů napadených samic lýkožroutů (THONG, WEBSTER 1975). Vliv přítomnosti běžných druhů endoparazitických hlístic *I. typographus* na letovou aktivitu nebyl prokázán (FORSSE 1987). Determinace jednotlivých druhů je obtížná, protože se uvnitř těla lýkožroutů objevují často pouze juvenilní jedinci a je nutné je dochovávat; např. larvy rodu *Parasitorhabditis* nelze od sebe rozeznat vůbec (RÜHM 1956).

Foretické druhy hlístic (např. rody *Diplogasteroides*, *Ditylenchus* a *Ektaphelenchus*) se nacházejí hlavně pod krovkami brouků, na křídlech či v prostorech mezi tělními články (RÜHM 1956) a prozatím nebyl prokázán žádný vliv na brouky.

Zelené řasy

U lýkožroutů rodu *Ips* nebyla nákaza touto skupinou zatím zaznamenána, ale lze ji předpokládat, protože byla zjištěna např. patogenní řasa *Helicosporidium* sp. (Chlorophyta: Trebouxiophyceae) u larev i dospělců *Dendroctonus micans* (Kugelann, 1794) v Turecku s průměrnou hladinou infekce 9 % (YAMAN 2008). Její využití v biologickém boji s *D. micans* komplikuje zjištění stejné infekce u přirozeného specifického predátora tohoto kůrovce - lesknáčka *Rhizophagus grandis* (Gyllenhal, 1827) (YAMAN, RADEK 2007; YAMAN et al. 2009), který je efektivním regulátorem a používá se v praxi již několik desítek let v euroasijském areálu (GRÉGOIRE et al. 1985; FIELDING et al. 1991; FIELDING, EVANS 1997).

Diskuse a Závěr

V současné době bylo popsáno více než 20 druhů patogenů (mikrosporidie, viry, prvoci, zelené řasy) u brouků podčeledi Scolytinae (WEGENSTEINER, WEISER 1996a, 2004; HÄNDEL et al. 2003). Většina autorů se jimi zabývala především z faunistického hlediska (WEISER et al. 1998; WEGENSTEINER 2004), a tak u většiny patogenů neznáme vlivy na brouky: na vitalitu, fertilitu, letové schopnosti, přezimování atd. Nejpodrobněji popsané druhové složení patogenů má lýkožrout smrkový *I. typographus*. V Evropě bylo zjištěno 10 onemocnění tohoto druhu (tab. 1); u některých dalších druhů, jako je např. *Ips cembrae* a *Ips amitinus*, nemáme dostatečné údaje, nebo dokonce donedávna nebyly vůbec žádné (HOLUŠA et al. 2012).

Mikrosporidie *Chytridiopsis typographi* a eugregarina *Gregarina typographi* jsou široce rozšířeny u společně se vyskytujících lýkožroutů rodu *Ips* (tab. 1), další druhy jsou odlišeny dle jednotlivých hostitelů a zahrnují řadu potencionálně nových druhů (HÄNDEL et al. 2003). Infekční hladina patogenů je do jisté míry závislá na populační hustotě a času, protože nákaza matečných brouků se může od tvorby snubních komůrek po dokončení vývinu potomstva i ztrojnásobit (LUKÁŠOVÁ, HOLUŠA 2011).

Infekční hladiny patogenů mohou záviset na populačních hustotách lýkožroutů. Při nízkých populačních denzitách se lýkožrouti setkávají s jedinci mimo svůj požerek jen velmi zřídka a spory patogenů tak nemohou být pozřeny s trusem nebo zbytky odumřelých těl a infikovat další jedince (WEGENSTEINER, WEISER 1996a).

Často bývají studovány lokality, kde je díky potencionální mobilitě brouků (běžně až 1 km) (WERMELINGER 2004) zabráněno vzniku subpopulací s jiným složením patogenů (HÄNDEL et al. 2003) a infekční hladiny i složení patogenů jsou konstantní i na poměrně vzdálených lokalitách (HOLUŠA et al. 2009). To vysvětluje mimo jiné i fakt, že v některých výzkumech bylo prokázáno, že hladiny patogenů nezávisejí na objemu napadených stromů (HOLUŠA et al. 2009) nebo že různé hladiny infekce nekorelují s různými počty brouků (WEGENSTEINER, WEISER 1996a).

Jedním z hlavních faktorů ovlivňujících populace je lesnický management. Předpokládáme, že kvůli absenci lesnického managementu a náhodné distribuci stresovaných stromů dochází ke shromáždění brouků a tím pádem ke vzniku ohnisek vysokých populačních hustot brouků a intenzivního předávání patogenů (HOLUŠA et al. 2009). Když je hustota lýkožroutů vysoká, mají nedospělí brouci nedostatek prostoru pro zralostní žír a pokračují v žíru skrz protínáním dalších požerků a larválních chodeb. To zvyšuje pravděpodobnost pozření patogenních spor, které byly uvolněny s trusem nakaženého jedince či jeho odumřením a rozkladem v místě požerku – infekční hladina patogenů se navyšuje (WEGENSTEINER, WEISER 1996a). Skupiny napadených stromů vytvářejí ohniska šíření lýkožroutů i jejich patogenů, jako je tomu např. na Šumavě či v Rakousku, kde se vyskytuje *ItEPV* a *Ch. typographi* ve velmi vysokých infekčních hladinách (WEGENSTEINER et al. 1996; WEISER et al. 2000; HOLUŠA et al. 2007).

Naopak infikovaní lýkožrouti v dobře obhospodařovaných lesích jsou více rozptýleni, protože lesníci rychle odstraňují napadené stromy. Odstranění stromů silně napadených kůrovci brání jednotlivým druhům patogenů v namnožení a nahromadění, které se objevuje v lesních porostech bez managementu, a vede k výraznému snížení některých patogenů či dokonce k jejich zániku na některých lokalitách. To se týká především patogenů lokalizovaných v tukovém tělese (např. *N. typographi* a *M. chalcographi*) (HOLUŠA et al. 2007, 2009).

S přihlédnutím k předběžným výsledkům celosvětových výzkumů je jasné, že patogeny nejsou schopny dostatečně a úspěšně regulovat populace lýkožroutů rodu *Ips*. Hlavní příčinou je komplikované aplikace v praxi (kultivace patogenů, kryptický způsob života kůrovců, v případě nízkých populačních hustot také téměř nulový přenos patogenů). Patogeny lýkožroutů rodu *Ips* nebudou v nejbližších letech použitelné jako jedno s obranných opatření proti těmto závažným škůdcům v lesních porostech.

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LITERATURA

BAKKE A. 1968. Ecological studies on bark beetles (Coleoptera: Scolytidae) associated with Scots pine (*Pinus sylvestris* L.) in Norway with particular reference to the influence of temperature. Meddelelser fra Norsk Institutt for Skogforskning, 21: 443-602.

BARTA J. R., THOMPSON R. C. 2006. What is *Cryptosporidium*? Reappraising its biology and phylogenetic affinities. Trends in Parasitology, 22: 463-468.

BATHON H. 1996. Impact of entomopathogenic nematodes on non-target hosts. Biocontrol Science and Technology, 6: 421-434.

BIDOCHKA M. J., MENZIES F. V., KAMP A. M. 2002. Genetic groups of the insect-pathogenic fungus *Beauveria bassiana* are associated with habitat and thermal growth preferences. Archives of Microbiology, 178: 531-537.

BOHNE W., FERGUSON D. J., KOHLER K., GROSS U. 2000. Developmental expression of a tandemly repeated, glycine- and serine-rich spore wall protein in the microsporidian pathogen *Encephalitozoon cuniculi*. Infection and Immunity, 68: 2268-2275.

BROSSON D., KUHN L., PRENSIER G., VIVARES C. P., TEXIER C. 2005. The putative chitin deacetylase of *Encephalitozoon cuniculi*: a surface protein implicated in microsporidian spore-wall formation. Fems Microbiology Letters, 247: 81-90.

BURJANADZE M., GOGINASHVILI N. 2009. Occurrence of pathogens and nematodes in the spruce bark beetles, *Ips typographus* (Col., Scolytidae) in Borjomi gorge. Bulletin of the Georgian National Academy of Sciences, 3: 145-149.

CABI/EPPO 1997. Quarantine Pests for Europe. In: Smith I. M. et al. (eds.). Wallingford, CAB International: 1425 s.

CALI A., TAKVORIAN P. 1999. Developmental morphology and life cycles of the microsporidia. In: Wittner M., Weiss L. (eds.): The microsporidia and microsporidiosis. Washington DC, American Society of Microbiology: 85-128.

CERYNGIER P., HODEK I. 1996. Enemies of Coccinellidae. In: Hodek I., Honěk A. (eds.): Ecology of Coccinellidae. Boston, Kluwer Academic Publishers: 319-350.

CLOPTON R. E. 2000 Phylum Apicomplexa Levine, 1970: order Eugregarinorida Léger, 1900. In: Lee J. J. et al. (eds.): Illustrated guide to the protozoa. Lawrence, Society of Protozoologists: 205-288.

CLOPTON R. E., GOLD R. E. 1996. Host specificity of *Gregarina blattarum* von Siebold, 1839 (Apicomplexa: Eugregarinida) among five species of domiciliary cockroaches. Journal of Invertebrate Pathology, 67: 219-223.

CORRADI N., KEELING P. J. 2009. Microsporidia: a journey through radical taxonomical revisions. Fungal Biology Reviews, 23: 1-8.

CROLL N. A. 1970. The behavior of nematodes. New York, St. Martin's Press: 117 s.

DELBAC F., POLONAIS V. 2008: The microsporidian polar tube and its role in invasion. Sub-cellular Biochemistry, 47: 208-220.

DRAGANOVA S., TAKOV D., DOYCHEV D. 2007. Bioassays with isolates of *Beauveria bassiana* (Bals.) Vuill. and *Paecilomyces farinosus* (Holm.) Brown, Smith against *Ips sexdentatus* Boerner and *Ips acuminatus* Gyll. (Coleoptera: Scolytidae). Plant Science, 44: 24-28.

DUNCAN L. W., MCCOY C. W. 1996. Vertical distribution in soil, persistence, and efficacy against citrus root weevil (Coleoptera: Curculionidae) of two species of entomogenous nematodes (Rhabditida: Steinernematidae: Heterorhabditidae). Environmental Entomology, 25: 174-178.

FIELDING N. J., O'KEEFE T., KING C. J. 1991. Dispersal and host-finding capability of the predatory beetle *Rhizophagus grandis* Gyll. (Col: Rhizophagidae). Journal of Applied Entomology, 112: 89-98.

FIELDING N. J., EVANS H. F. 1997. Biological control of *Dendroctonus micans* (Scolytidae) in Great Britain. Biocontrol, 18: 51-60.

FORSSE E. 1987. Flight duration in *Ips typographus* L.: insensitivity to nematode infection. Journal of Applied Entomology, 104: 326-328.

FORST S., DOWDS B., BOEMARE N., STACKEBRANDT E. 1997. *Xenorhabdus* and *Photorhabdus* spp.: bugs that kill bugs. Annual Review of Microbiology, 51: 47-72.

FRANZEN C. 2004. Microsporidia: how can they invade other cells? Trends in Parasitology, 20: 275-279.

FRANZEN C. 2005. How do microsporidia invade cells? Folia Parasitologica, 52: 36-40.

FUCHS G. 1915. Die Naturgeschichte der Nematoden und einiger anderer Parasiten 1. des *Ips typographus* L. 2. des *Hylobius abietis* L. Zoologische Jahrbücher, Abteilung für Systematik, 38: 109-222.

GAUGLER R. 2002. Entomopathogenic Nematology. Wallingford, CABI Publishing: 372 s.

GAUGLER R., KAYA H. K. 1990. Entomopathogenic nematodes in biological control. Boca Raton, CRC Press: 365 s.

GRÉGOIRE J.-C., MERLIN J., PASTEELS J. M., JAFFUELS R., VOULAND G., SCHVESTER D. 1985. Biocontrol of *Dendroctonus micans* by *Rhizophagus grandis* in the Massif Central (France): a first appraisal of the mass-rearing and release methods. Zeitschrift für Angewandte Entomologie, 99: 182-190.

GRODZKI W. 1997. Possibilities of the control of the double-spined bark beetle *Ips duplicatus* C. R. Sahlb in the Southern Poland. Sylwan, 11: 25-36.

GRODZKI W. 2003. Distribution range of the double spined bark beetle *Ips duplicatus* C. R Sahlb (Col.: Scolytidae) in the mountain areas of southern Poland. Sylwan, 8: 29-36.

HAJEK A.E., ST. LEGER R.J. 1994. Interactions between fungal pathogens and insect hosts. Annual Review of Entomology, 39: 293-322.

HÄNDEL U., KENIS M., WEGENSTEINER R. 2001. Untersuchungen zum Vorkommen von Pathogenen und Parasiten in Populationen überwinternder Fichtenborkenkäfer (Col., Scolytidae). Mitteilungen der Deutschen Gesellschaft für allgemeine und angewandte Entomologie, 13: 423-428.

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.

HAYMAN J. R., HAYES S. F., AMON J., NASH T. E. 2001. Developmental expression of two spore wall proteins during maturation of the microsporidian *Encephalitozoon intestinalis*. Infection and Immunity, 69: 7057-7066.

HOLUŠA J., ZAHRADNÍK P., KNÍŽEK M., DRÁPELA K. 2003. Seasonal flight activity of the double-spined spruce bark-beetle *Ips duplicatus* (Coleoptera, Curculionidae, Scolytinae) in Silesia (Czech Republic). Biologia, 58: 935-941.

HOLUŠA J., WEISER J., PULTAR O. 2004. Possibility of using of virus against bark beetles? Experiences with simulated infection of two bark beetles, *Ips typographus* and *Ips duplicatus*, with three pathogens. In: Csóka G. et al. (eds.): Biotic damage in forests. Proceedings of the IUFRO Symposium (WP 7. 03. 10 "Methodology of forest pest and disease survey in Central Europe"). Mátrafüred, Hungary, September 12.-16., 2004. Mátrafüred, Hungarian Forest Research Institute: 72-78.

HOLUŠA J., WEISER J. 2005. Biologické postupy boje s lesními škůdci. Zpravodaj ochrany lesa, 11: 18-23.

HOLUŠA J., WEISER J., DRÁPELA K. 2007. Pathogens of *Ips duplicatus* (Coleoptera, Scolytidae) in three areas in Central Europe. Acta Protozoologica, 46: 157-167.

HOLUŠA J., WEISER J., ŽIŽKA Z. 2009. Pathogens of the spruce bark beetles *Ips typographus* and *Ips duplicatus*. Central European Journal of Biology, 4: 567-573.

HOLUŠA J., LUKÁŠOVÁ K., WEGENSTEINER R., GRODZKI W., PERNEK M., WEISER J. 2012. Pathogens of the bark beetle *Ips cembrae*: microsporidia and gregarines known from other *Ips* species. Journal of Applied Entomology [in press].

KAYA H. K. 1984. Nematode parasites of bark beetles. In: Nickle W. R. (eds.): Plant and insect nematodes. New York, Marcel Dekker, Inc.: 727-754.

KAYA H. K., GAUGLER R. 1993. Entomopathogenic nematodes. Annual Review of Entomology, 38: 181-206.

KERESELIDZE M., WEGENSTEINER R., GOGINASHVILI N., TVARADZE M., PILARSKA D. 2010. Further studies on the occurrence of natural enemies of *Ips typographus* (Coleoptera: Curculionidae: Scolytinae) in Georgia. Acta Zoologica Bulgarica, 62: 131-139.

KIRCHHOFF J.-F., FÜHRER E. 1990. Experimentelle Analyse der Infektion und des Entwicklungszyklus von *Malamoeba scolyti* in *Dryocoetes autographus* (Coleoptera: Scolytidae). Entomophaga, 35: 537-544.

KOEHLER P.G., PATTERSON R.S., MARTIN W.R. 1992. Susceptibility of cockroaches (Dictyoptera: Blattellidae, Blattidae) to infection by *Steinernema carpocapsae*. Journal of Economic Entomology, 85: 1184-1187.

KREUTZ J., VAUPEL O., ZIMMERMANN G. 2004. 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.

LANDA Z., HORŇÁK P., OSBORNE L. S., 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.

LANDA Z., KŘENOVÁ Z., VOJTĚCH O. 2007. Využití houby *Beauveria bassiana* v ochraně proti lýkožroutu smrkovému. Lesnická práce, 86: 646-647.

LANGE C.E., WITTENSTEIN E. 2002. The life cycle of *Gregarina ronderosi* n. sp. (Apicomplexa: Gregarinidae) in the Argentine grasshopper *Dichroplus elongatus* (Orthoptera: Acrididae). Journal of Invertebrate Pathology, 79: 27-36.

LIEUTIER F. 1980. Le parasitisme d'*Ips sexdentatus* (Boern) (Coleoptera: Scolytidae) par les nématodes du genre *Parasitaphelenchus* Fuchs. Relations avec le parasitisme par *Contortylenchus diplogaster* (v. Lins.). Revue Nématology, 3: 271-281.

LIPA J. J. 1967. Studies on gregarines (Gregarinomorpha) of arthropods in Poland. Acta Protozoologica, 5: 97-179.

LOCKLIN J. L., VODOPICH D. S. 2010. Patterns of gregarine parasitism in dragonflies: host, habitat, and seasonality. Parasitology Research, 107: 75-87.

LUKÁŠOVÁ K., HOLUŠA J. 2011. *Gregarina typographi* (Eugregarinorida: Gregarinidae) in the bark beetle *Ips typographus* (Coleoptera: Curculionidae): changes in infection level in the breeding system. Acta Protozoologica, 50: 311-318.

MAZUR A., ŁABĘDZKI A., RAJ A. 2006. Observations of phenology and frequencing of the spruce bark beetle (*Ips typographus* L.) and its accompanying species in the uppermost subalpine spruce forest zone environment in the Karkonoski National Park. In: Kolk A. (ed.): Insect outbreaks in managed and unmanaged forests. Warszawa, Instytut Badawczy Leśnictwa: 29-48.

MICHALKOVÁ V., KRASCSENITSOVÁ E., KOZÁNEK M. 2011. On the pathogens of the spruce bark beetle *Ips typographus* (Coleoptera: Scolytinae) in the Western Carpathians. Biologia, 67: 217-221.

MRÁČEK Z. 1986. Nematodes and other factors controlling the sawfly, *Cephaleia abietis* (Pamphilidae, Hymenoptera), in Czechoslovakia. Forest Ecology and Management, 15: 75-79.

MRÁČEK Z., BEČVÁŘ S. 2000. Insect aggregations and entomopathogenic nematode occurrence. Nematology, 2: 297-301.

OEPP/EPPO 2005. *Ips cembrae* and *Ips subelongatus*. Bulletin OEPP/EPPO, 35: 445-449.

OMOTO C. K., TOSO M., TANG K., SIBLEY L. D. 2004. Expressed sequence tag (EST) analysis of gregarine gametocyst development. International Journal for Parasitology, 34: 1265-1271.

PERKINS F. O. 2000. Order Neogregarinorida Grassé, 1953. In: Lee J. J. et al. (eds.): The illustrated guide to the Protozoa. Lawrence, Society of Protozoologists: 288-298, 353-369.

PEUVEL-FANGET I., POLONAIS V., BROSSON D., TEXIER C., KUHN L., PEYRET P., VIVARES C. P., DELBAC F. 2006. EnP1 and EnP2, two proteins associated with the *Encephalitozoon cuniculi* endospore, the chitin-rich inner layer of the microsporidian spore wall. International Journal for Parasitology, 36: 309-318.

PFEFFER A., KNÍŽEK M. 1995. Expanze lýkožrouta *Ips duplicatus* (Sahlb.) ze severské tajgy. Zpravodaj ochrany lesa, 2: 8-11.

PHELPS N. B. D., GOODWIN A. E. 2008. Vertical transmission of *Ovipleistophora ovariae* (Microspora) within the eggs of the Golden Shiner. Journal of Aquatic Animal Health, 20: 45-53.

POINAR G. O., DESCHAMPS N. 1981. Susceptibility of *Scolytus multistriatus* to neoaplectanid and heterorhabditid nematodes. Environmental Entomology, 10: 85-87.

POSTNER M. 1974. *Ips cembrae*. In: Schwenke W. (ed.): Die Forstschädlinge Europas. II. Band. Käfer. Hamburg, Paul Parey: 458-459.

PULTAR O., WEISER J. 2004: Výsledky posledních patologických studií dominantních kůrovců v NP Šumava a jejich využití. In: Sborník referátů 28. setkání lesníků tří generací na téma „Nebezpečí kůrovce v roce 2004" 19. 3. 2004 (Praha - Novotného lávka). Česká lesnická společnost, VÚLHM Jíloviště-Strnady, Lesnická práce: 82-88.

PURRINI K. 1978. Protozoen als Krankheitserreger bei einigen Borkenkäferarten (Col., Scolytidae) im Königsee-Gebiet, Oberbayern. Anzeiger für Schädlingskunde, Pflanzenschutz, Umweltschutz, 51: 171-175.

PURRINI K., FÜHRER E. 1979. Experimentelle Infection von *Pityogenes chalcographus* L. (Coleoptera, Scolytidae) durch *Malamoeba scolyti* Purrini (Amoebina, Amoebidae) und *Menzbieria chalcographi* Weiser (Neogregarina, Ophryocystidae). Anzeiger für Schädlingskunde, Pflanzenschutz und Umweltschutz, 52: 167-173.

PURRINI K., ŽIŽKA Z. 1983. More on the life cycle of *Malamoeba scolyti* (Amoebidae: Sarcomastigophora) parasitizing the bark beetle *Dryocoetes autographus* (Scolytidae, Coleoptera). Journal of Invertebrate Pathology, 42: 96-105.

RAMOS-RODRÍGUEZ O., CAMPBELL J. F., RAMASWAMY S. B. 2006. Pathogenicity of three species of entomopathogenic nematodes to some major stored-product insect pests. Journal of Stored Products Research, 42: 241-252.

REDHEAD S. A., KIRK P. M., KEELING P. J., WEISS L. M. 2009. Proposals to exclude the phylum Microsporidia from the Code. Mycotaxon, 108: 505-507.

ROBERTS D. W., HAJEK A. E. 1992. Entomopathogenic fungi as bioinsecticides. In: Leatham G. F. (ed.): Frontiers of industrial mycology. New York, Chapman and Hall: 144-159.

RÜHM W. 1956. Die Nematoden der Ipiden. Mit 10 Tabellen im Text. Jena, Fischer: 437 s.

SHANNAG H. K., CAPINERA J. L. 2000. Interference of *Steinernema carpocapsae* (Nematoda: Steinernematidae) with *Cardiochiles diaphaniae* (Hymenoptera: Braconidae), a parasitoid of melonworm and pickleworm (Lepidoptera: Pyralidae). Environmental Entomology, 29: 612-617.

SCHAWANG J. E., JANOVY J. JR. 2001. The response of *Gregarina niphandrodes* (Apicomplexa: Eugregarinida: Septatina) to host starvation in *Tenebrio molitor* (Coleoptera: Tenebrionidae) adults. Journal of Parasitology, 87: 600-605.

SHAPIRO D. I., MCCOY C. W. 2000. Virulence of entomopathogenic nematodes to *Diaprepes abbreviatus* (Coleoptera: Curculionidae) in the laboratory. Journal of Economic Entomology, 93: 1090-1095.

SIENKIEWICZ P., LIPA J. 2008. *Gregarina vizri* Lipa, 1968 (Apicomplexa: Eugregarinida) recorded in Poland in an expansive plant pest the cereal ground beetle *Zabrus tenebrioides* (Goeze) (Coleoptera: Carabidae). Journal of Plant Protection Research, 48: 189-193.

SIMMONS L. W. 1990. Post-copulatory guarding female choice and the levels of gregarine infections in the field cricket *Gryllus binzaculatus*. Behavioral Ecology and Sociobiology, 26: 403-407.

SMITH A.J., COOK T.J., LUTTERSCHMIDT W.I. 2007. Effects of temperature on the development of *Gregarina cubensis* (Apicomplexa: Eugregarinida) parasitizing *Blaberus discoidalis* (Blattaria: Blaberidae). Journal of Parasitology, 93: 583-588.

SMITH A.J., COOK T.J. 2008. Host specificity of five species of Eugregarinida among six species of cockroaches (Insecta: Blattodea). Comparative Parasitology, 75: 288-291.

TAKOV D., PILARSKA D., WEGENSTEINER R. 2006. Entomopathogens in *Ips typographus* (Coleoptera: Scolytidae) from several spruce stands in Bulgaria. Acta Zoologica Bulgarica, 58: 409-420.

TAKOV D., DOYCHEV D., WEGENSTEINER R., PILARSKA D. 2007. Study on the pathogens of bark beetles (Coleoptera, Scolytidae) from different coniferous stands in Bulgaria. Acta Zoologica Bulgarica, 59: 87-96.

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.

TAKOV D., DOYCHEV D., LINDE A., DRAGANOVA S., PILARSKA D. 2011. Pathogens of bark beetles (Coleoptera: Curculionidae) in Bulgarian forests. Phytoparasitica, 39: 343-352.

TANADA Y., KAYA H. K. 1993. Protozoan infection: Apicomplexa, Microspora. In: Insect Pathology. San Diego, Academic Press: 414-458.

THÉODORIDÉS J. 1984. The phylogeny of the Gregarinia (Sporozoa). Origins of Life and Evolution of Biospheres, 13: 339-342.

THONG C. H. S., WEBSTER J. M. 1975. Effects of bark beetle nematode, *Contortylenchus reversus*, on gallery construction, fecundity, and egg viability of douglas-fir beetle, *Dendroctonus pseudotsugae* (Coleoptera-Scolytidae). Journal of Invertebrate Pathology, 26: 235-238.

TONKA T., WEISER J. JR., WEISER J. 2010. Budding: A new stage in the development of *Chytridiopsis typographi* (Zygomycetes: Microsporidia). Journal of Invertebrate Pathology, 104: 17-22.

TOSO M., OMOTO C. K. 2007. Ultrastructure of *Gregarina niphandrodes* nucleus through stages from unassociated trophozoites to gamonts in syzygy and the syzygy junction. Journal of Parasitology, 93: 479-484.

TRONCHIN G., SCHREVEL J. 1977. Chronologie des modifications ultrastructurales au cours de la croissance de *Gregarina blaberae*. Journal of Protozoology, 24: 67-82.

UNAL S., YAMAN M., TOSUN O., AYDIN C. 2009. Occurence of *Gregarina typographi* (Apicomplexa, Gregarinidae) and *Metschnikowia typographi* (Ascomycota, Metschnikowiaceae) in *Ips sexdentatus* (Coleoptera: Curculionidae, Scolytinae) populations in Kastamonu (Turkey). Journal of Animal and Veterinary Advances, 8: 2687-2691.

VALIGUROVÁ A., MICHALKOVÁ V., KOUDELA B. 2009. Eugregarine trophozoite detachment from the host epithelium via epimerite retraction: fiction or fact? International Journal for Parasitology, 39: 1235-1242.

VANNINEN I. 1996. Distribution and occurence of four entomopathogenic fungi in Finland: effect of geographical location, habitat types and soil type. Mycological Research, 100: 93-101.

VÁVRA J., LARSSON J.I.R. 1999. Structure of the microsporidia. In: Whittner M., Weiss L.M. (eds.): The microsporidia and microsporidiosis. Washington, D.C., ASM Press: 7-84.

WEGENSTEINER R. 1994. *Chytridiopsis typographi* (Protozoa, Microsporidia) and other pathogens in *Ips typographus* (Coleoptera, Scolytidae). IOBC/WPRS Bulletin, 17: 39-42.

WEGENSTEINER R., WEISER J. 1995. A new *Entomopoxvirus* in the bark beetle *Ips typographus* (Coleoptera: Scolytidae). Journal of Invertebrate Pathology, 65: 203-205.

WEGENSTEINER R. 1996. Laboratory evaluation of *Beauveria bassiana* (Bals.) Vuill. against *Ips typographus* (Coleoptera, Scolytidae). IOBC/WPRS Bulletin, 19: 186-189.

WEGENSTEINER R., WEISER J., FÜHRER E. 1996. Observations on the occurrence of pathogens in the bark beetle *Ips typographus* L. (Coleoptera, Scolytidae). Journal of Applied Entomology, 120: 199-204.

WEGENSTEINER R., WEISER J. 1996a. Occurrence of *Chytridiopsis typographi* (Microspora, Chytridiopsida) in *Ips typographus* L. (Col., Scolytidae) field population and in a laboratory stock. Journal of Applied Entomology, 120: 595-602.

WEGENSTEINER R., WEISER J. 1996b. Untersuchungen zum Auftreten von Pathogen by *Ips typographus* (Coleoptera: Scolytidae) aus einem Naturschutzgebiet in Schwartzwald (Baden-Württemberg). Anzeiger für Schädlingskunde, Pflanzenschutz, Umweltschutz, 69, 162-167.

WEGENSTEINER R., WEISER J. 2004. Annual variation of pathogen occurrence and pathogen prevalence in *Ips typographus* L. (Col, Scolytidae) from the BOKU University Forest Demonstration Centre. Journal of Pest Science, 77: 221-228.

WEGENSTEINER R. 2004. Pathogens in bark beetles. In: Lieutier F. et al. (eds.): Bark and wood boring insects in living trees in Europe: a synthesis. Dordrecht, Kluwer: 291-313.

WEGENSTEINER R., DEDRYVER C.-A., PIERRE J.-S. 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.

WEISER J. 1966. Nemoci hmyzu. Praha, Academia: 556 s.

WEISER J., MRÁČEK Z. 1988. Parazitické hlístice hmyzu. Praha, Academia: 260 s.

WEISER J., WEGENSTEINER R. 1994. A new *Entomopoxvirus* in the bark beetle *Ips typographus* (Coleoptera, Scolytidae) in Czechoslovakia. Zeitschrift für angewandte Zoologie, 80: 425-434.

WEISER J., WEGENSTEINER R., ŽIŽKA Z. 1995. *Canningia spinidentis* gen. et sp. n. (Protista: Microspora), a new pathogen of the fir bark beele *Pityokteines spinidens*. Folia Parasitologica, 42: 1-10.

WEISER J., WEGENSTEINER R., ŽIŽKA Z. 1998. *Unikaryon montanum* sp.n., (Protista, Microspora), a new pathogen of the spruce bark beetle, *Ips typographus* (Coleoptera: Scolytidae). Folia Parasitologica, 45: 191-195.

WEISER J., PULTAR O., ŽIŽKA Z. 2000. Biological protection of forest against bark beetle outbreaks with poxvirus and other pathogens. IUAPPA, Section B, 12: 168-172.

WEISER J. 2002. Patogenní organismy. In: Skuhravý V. (ed.): Lýkožrout smrkový (*Ips typographus* L.) a jeho kalamity. Praha, Agrospoj: 97-100.

WEISER J., WEGENSTEINER R., HÄNDEL U., ŽIŽKA Z. 2003. Infections with the Ascomycete *Metschnikowia typographi* n. sp. in the bark beetle *Ips typographus* and *Ips amitinus* (Col., Scolytidae.). Folia Microbiologica, 48: 611-618.

WEISER J., HOLUŠA J., ŽIŽKA Z. 2006. *Larssoniella duplicati* n.sp. (Microsporidia. Unikaryonidae), a newly described pathogen infecting the double-spined spruce bark beetle, *Ips duplicatus* (Coleoptera, Scolytidae) in the Czech Republic. Journal of Pest Science, 79: 127-135.

WERMELINGER B. 2004. Ecology and management of the spruce bark beetle *Ips typographus* – a review of recent research. Forest Ecology and Management, 202: 67-82.

WERMELINGER B., RIGLING A., SCHNEIDER MATHIS D., DOBBERTIN M. 2008. Assessing the role of bark- and wood-boring insects in the decline of Scots pine (*Pinus sylvestris*) in the Swiss Rhone valley. Ecological Entomology, 33: 239-249.

WITTNER M., WEISS L. M. 1999. The microsporidia and microsporidiosis. Washington DC, ASM Press: 553 s.

XU Y., WEISS L. M. 2005. The microsporidian polar tube: a highly specialised invasion organelle. International Journal for Parasitology, 35: 941-953.

YAMAN M. 2002. *Gregarina phyllotretae* Hoshide 1953, a protozoan parasite of the flea beetles, *Phyllotreta undulata* and *P. atra* (Coleoptera: Chrysomelidae) in Turkey. Applied Entomology and Zoology, 37: 649-653.

YAMAN M. 2007. *Gregarina typographi* Fuchs, a gregarine pathogen of the six-toothed pine bark beetle, *Ips sexdentatus* (Boerner) (Coleoptera: Curculionidae, Scolytinae) in Turkey. Turkish Journal of Zoology, 31: 359-363.

YAMAN M., RADEK R. 2007. Infection of the predator beetle *Rhizophagus grandis* Gyll. (Coleoptera, Rhizophagidae) with the insect pathogenic alga *Helicosporidium* sp. (Chlorophyta: Trebouxiophyceae). Biological Control, 41: 384-388.

YAMAN M. 2008. First results on distribution and occurrence of the insect pathogenic alga *Helicosporidium* sp. (Chlorophyta: Trebouxiophyceae) in the populations of the great spruce bark beetle, *Dendroctonus micans* (Kugelann) (Coleoptera: Curculionidae, Scolytinae). North-Western Journal of Zoology, 4: 99-107.

YAMAN M., RADEK R., AYDIN C., TOSUN O., ERTÜRK Ö. 2009. First record of the insect pathogenic alga *Helicosporidium* sp. (Chlorophyta: Trebouxiophyceae) infection in larvae and pupae of *Rhizophagus grandis* Gyll. (Coleoptera, Rhizophaginae) from Turkey. Journal of Invertebrate Pathology, 102: 182-184.

YAMAN M., BAKI H. 2011. First record of *Entomopoxvirus* of *Ips typographus* (Linnaeus) (Coleoptera: Curculionidae, Scolytinae) for Turkey. Acta Zoologica Bulgarica, 63: 199-202.

ZITTERER P. M. 2002. Antagonists of *Ips acuminatus* (Gyllenhall) with special consideration of pathogens. Diploma thesis. Wien, Universität für Bodenkultur: 56 s.

ZUK M. 1987. The effects of gregarine parasites, body size, and time of day on spermatophore production and sexual selection in field crickets. Behavioral Ecology and Sociobiology, 21: 65-72.

Tab. 1.

Přehled zjištěných patogenů (bez hlístic a hub s výjimkou mikrosporidií a *M. typographi*) u lýkožroutů rodu *Ips* (dle Wegensteiner 2004; Takov et al. 2006; Burjanadze, Goginashvili 2009; Unal et al. 2009; Takov et al. 2010; Wegensteiner et al. 2010; Lukášová, Holuša 2011; Michalková et al. 2011; Takov et al. 2011; Holuša et al. 2012)

Overview of the identified pathogens (excluding nematodes and fungi except microsporidia and *M. typographi*) on bark beetle genus *Ips* (according to Wegensteiner 2004; Takov et al. 2006; Burjanadze, Goginashvili 2009; Unal et al. 2009; Takov et al. 2010; Wegensteiner et al. 2010; Lukášová, Holuša 2011; Michalková et al. 2011; Takov et al. 2011; Holuša et al. 2012) (listed from the top: Pathogens/Species, Systematic classification, Σ citations, Σ pathogens).

Patogeny/Druhy	Systematické zařazení	*Ips typographus*	*Ips acuminatus*	*Ips sexdentatus*	*Ips amitinus*	*Ips duplicatus*	*Ips cembrae*	Σ citace
Malamoeba scolyti	Rhizopoda	x	x	-	-	-	-	6
*Gregarina typographi**	Apicomplexa	x	x	x	x	x	x	33
Telosporidium typographi	Apicomplexa	x	-	-	-	-	-	1
Menzbieria chalcographi	Apicomplexa	x	-	-	-	-	-	4
*Mattesia schwenkei**	Apicomplexa	x	x	-	-	-	x	7
Chytridiopsis typographi	Microsporidia	x	x	-	x	x	x	22
*Nosema typographi**	Microsporidia	x	-	-	-	-	x	10
*Unikaryon montanum**	Microsporidia	x	-	-	x	-	-	6
Larssoniella duplicati	Microsporidia	-	-	-	-	x	-	3
Metschnikowia typographi	Ascomycota	x	-	x	x	-	-	4
ItEPV	dsDNA viry	x	-	-	x	-	-	8
Σ patogeny		10	4	2	5	3	4	**104**

*Druhy, u kterých si autoři nebyli jistí a určili je pouze do rodu, jsme přiřadili k již popsaným druhům. Zřejmě se totiž jedná o tyto již existující druhy (vzhledem k podobné bionomii a vazbě na stejné hostitelské dřeviny lýkožroutů). Bez genetických testů, které jsou zejména u mikrosporidií velmi komplikované, není zatím potřeba je striktně rozlišovat.

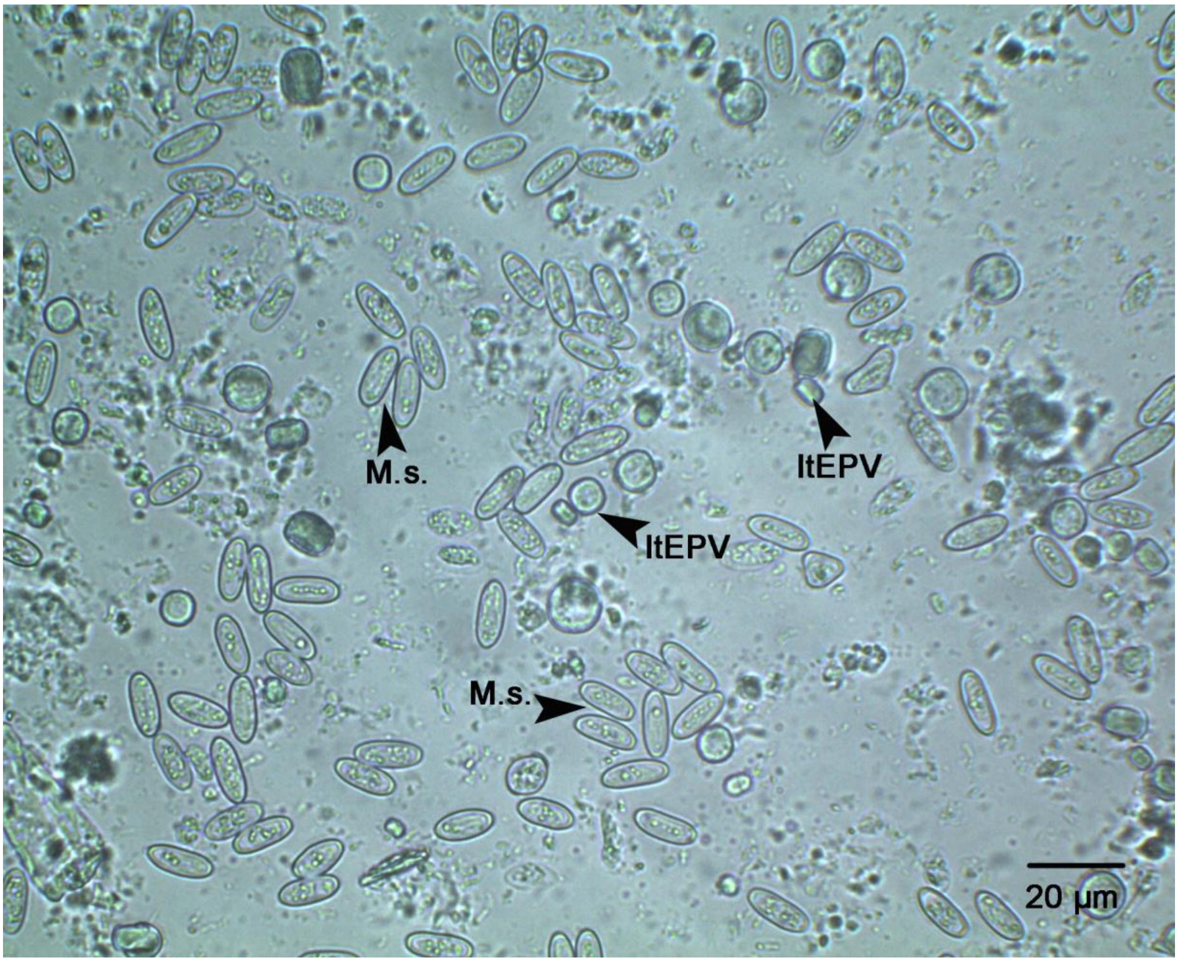
* Species of which the authors were not sure and determined only their genus, were assigned to the previously described species. Probably they are already existing species (considering similar bionomy and relation to the same host tree species of bark beetles). Without genetic tests that are especially in microsporidia very complicated, it is not strictly necessary to separate them yet.

Obr. 1.

Sferoidy *ItEPV* a cysty neogregariny *Mattesia schwenkei* (M.s.) v hemolymfě *Ips typographus*

Fig. 1.

Spheroids of *ItEPV* and cysts of neogregarine *Mattesia schwenkei* (M.s.) in the hemolymph of *Ips typographus*

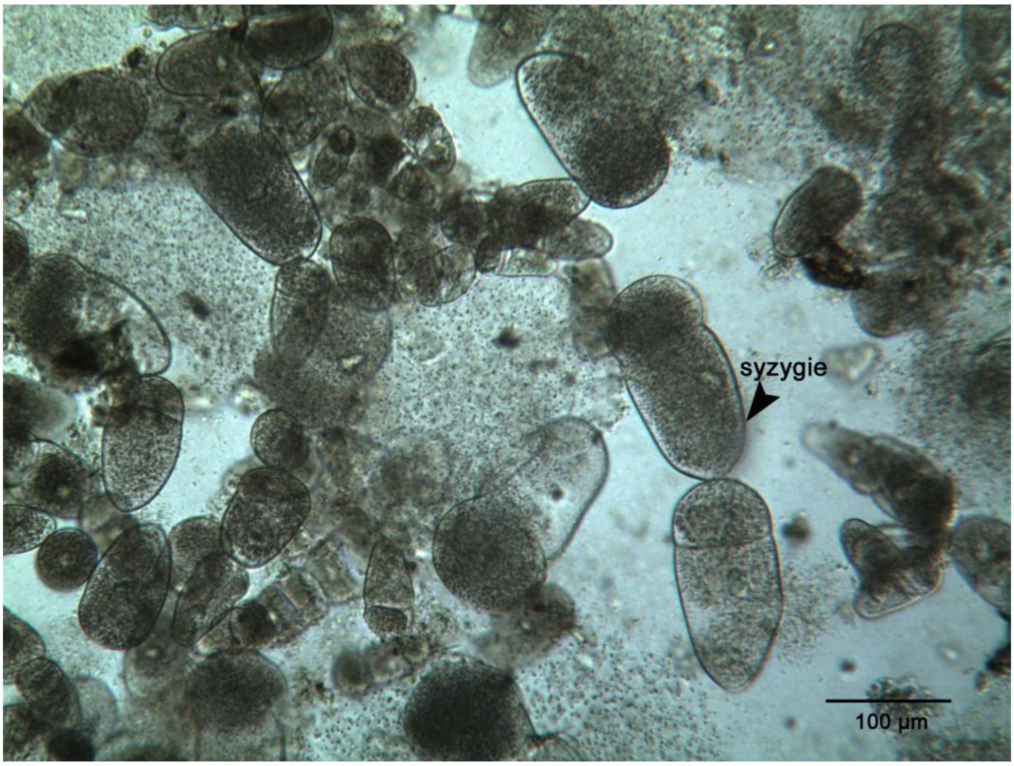


Obr. 2.

Stadium trofozoitů, gamontů a syzygie *Gregarina typographi* zaznamenaná u lýkožrouta *Ips typographus*

Fig. 2.

Life stage of trophozoites, gamonts and syzygy of *Gregarina typographi* recorded in bark beetle *Ips typographus*

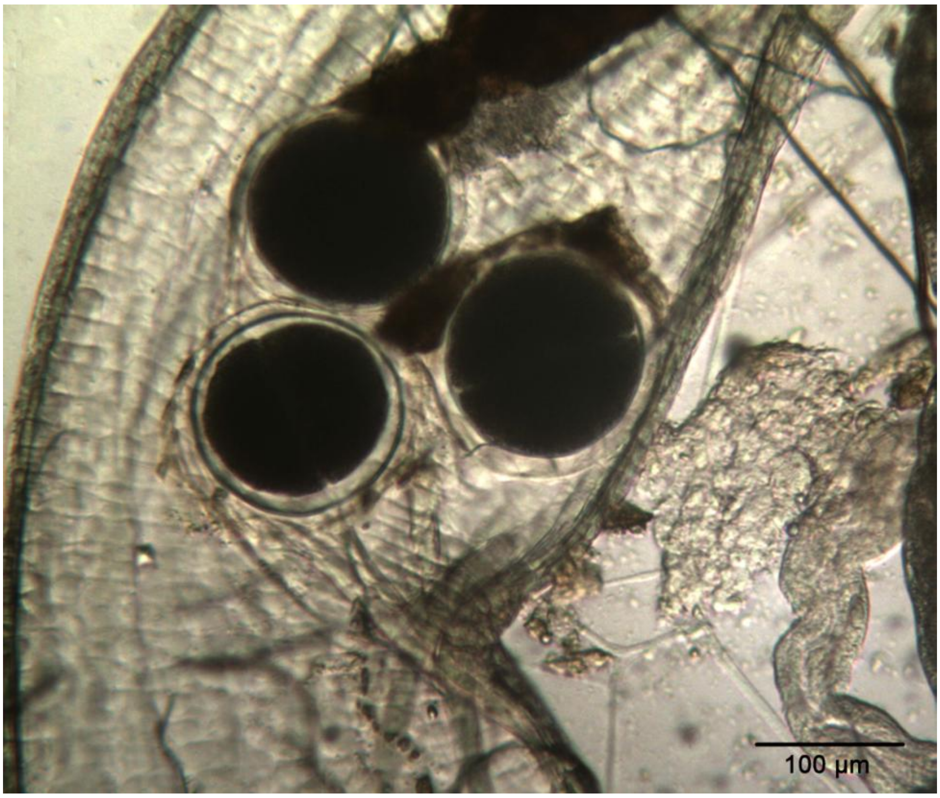


Obr. 3.

Gametocysty *Gregarina typographi* v lumenu střeva *Ips typographus*

Fig. 3.

Gametocysts of *Gregarina typographi* in gut lumen of *Ips typographus*

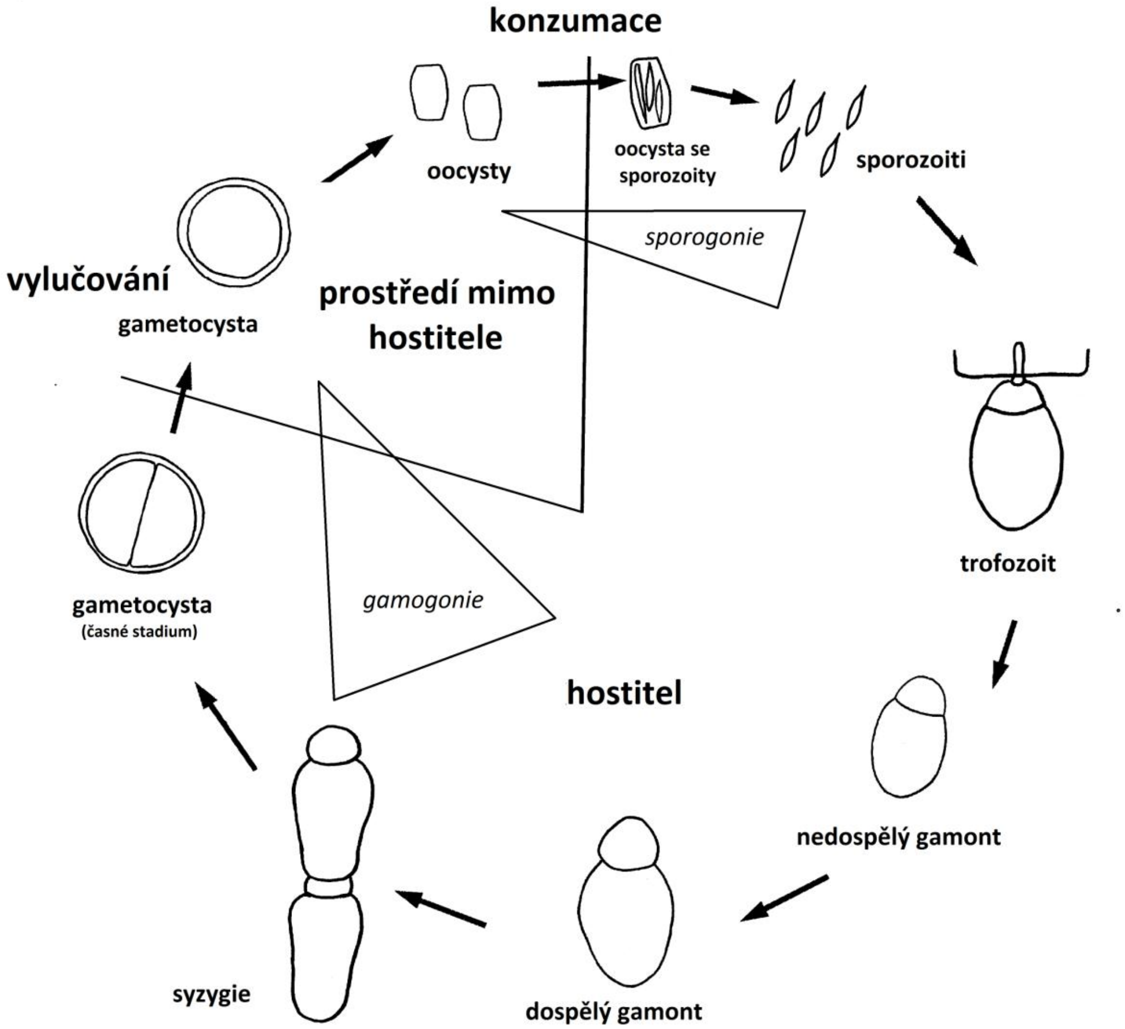


Obr. 4.

Životní cyklus *Gregarina typographi*

Fig. 4.

Life cycle of *Gregarina typographi*

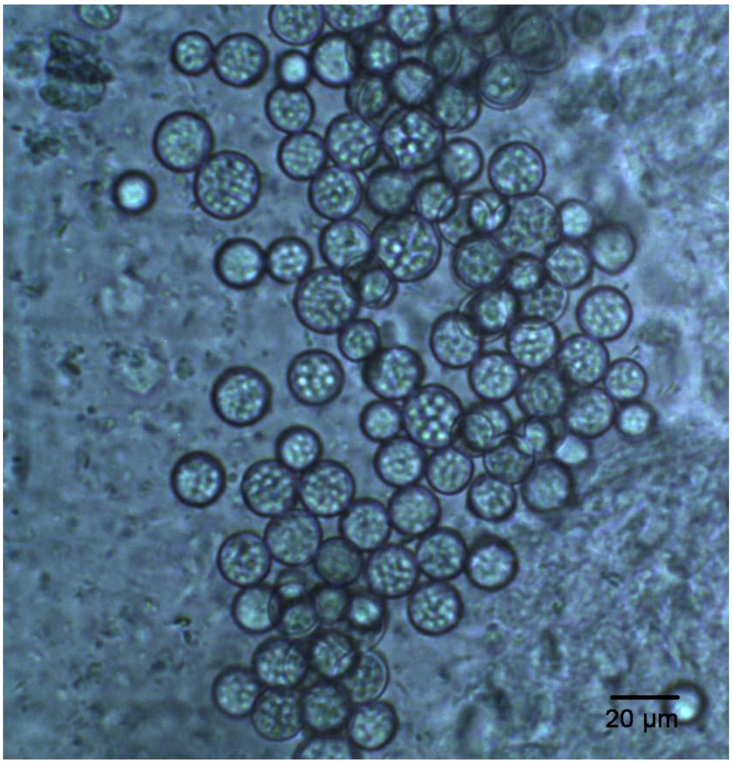


Obr. 5.

Silnostěnné cysty *Chytridiopsis typographi* nalezené v mesenteronu *Ips typographus*

Fig. 5.

Thick-walled cysts of *Chytridiopsis typographi* found in midgut of *Ips typographus*

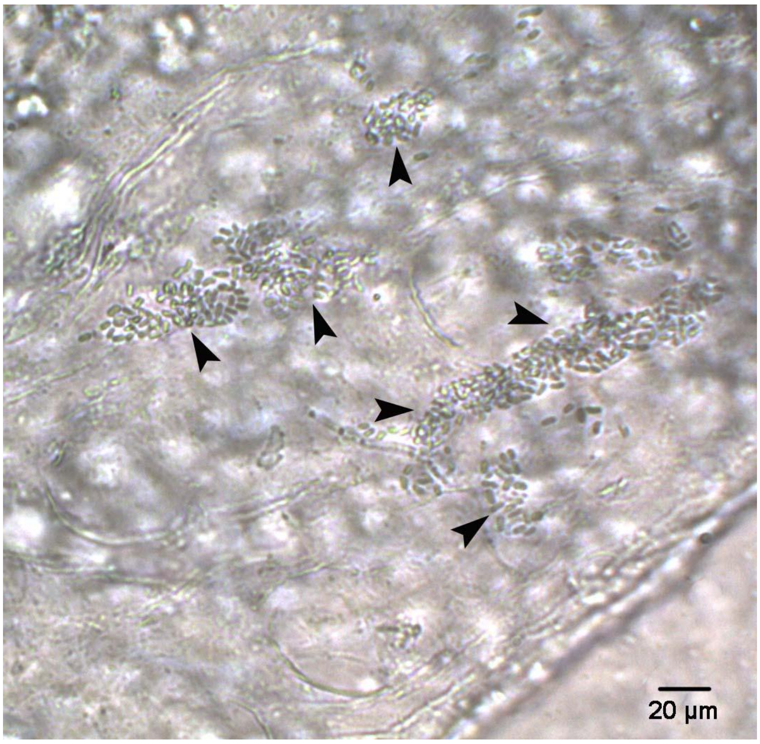


Obr. 6.

Povrch střeva se sporami *Larssoniella duplicati* ve svalových vláknech středního střeva *Ips duplicatus*

Fig. 6.

Surface of the midgut with spores of *Larssoniella duplicati* in the muscle strands of the muscularis in the midgut of *Ips duplicatus*



PATHOGENS OF BARK BEETLES OF THE GENUS *IPS* (COLEOPTERA: CURCULIONIDAE: SCOLYTINAE): REVIEW

SUMMARY

Bark beetles genus *Ips* (Coleoptera: Curculionidae: Scolytinae) are one of the most dangerous forest insect pests in Europe. At present, around twenty species of pathogens and entomopathogenic nematodes were described in the bark beetle subfamily Scolytinae. The most detailed species composition of pathogens has spruce bark beetle *Ips typographus* has ten species (Tab. 1). For some other bark beetle species such as *Ips amitinus* and *I. cembrae* no sufficient data are available about pathogens or have been recently studied. First pathogen in bark beetles were described early in the 20th century, most species were then identified in the second half of the 20th century. In 2006, the last known pathogen was described – microsporidia species-specific, *Larssoniella duplicati* from *I. duplicatus* found in the east part of the Czech Republic and northeastern Poland (Fig. 6). It is a chronic, widespread infection. Infection level of this pathogen reaches in some study sites up to 80%.

The only virus - Entomopoxvirus (*ItEPV*; Fig. 1) that was recorded in bark beetles causes the formation of protein inclusions only in the midgut of adult beetles. Microsporidia *Chytridiopsis typographi* (Fig. 5) and eugregarine *Gregarina typographi* (Fig. 2–4) are widely distributed in populations of bark beetle genus *Ips* (Tab. 1). Fat body of host insects are attacked and destroyed by neogregarines (*Menzbieria chalcographi* and *Mattesia schwenkei*) and some microsporidia (*Nosema typographi*). This disease causes high mortality of individuals during the overwintering period in the bark and forest litter.

For most pathogens no information is available about influences on populations of bark beetles. For example it has been shown that nematodes do not affect the flight activity of *Ips typographus*. However, knowledge of the nematodes of the genus *Ips* is still very limited and since the 1950s we have not had much new information.

Infection levels of pathogens may depend on the bark beetle population densities. At low population density callow beetle have contact with individuals outside their breeding system very rarely and pathogens such as spores cannot be ingested with faeces or remains of dead bodies of infected individuals. We assume that the absence of forest management leads to

high population density of beetles on host trees and thus to the intensive transmission of pathogens.

Comprehensively none of the previously identified pathogenic organisms has been ever demonstrated as an effective biological control agent. Although laboratory experiments are often successful (f. e. with *Beauveria bassiana* or *Malamoeba scolyti*), cryptic life cycle of genus *Ips* under the bark and their bionomy in general is still a major complication in the succesfull aplication of biological control.

Recenzováno

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4.2 Is *Ips amitinus* (Coleoptera: Curculionidae) abundant in wide range of altitudes?

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Is *Ips Amitinus* (Coleoptera: Curculionidae) Abundant in Wide Range of Altitudes?

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Abstract: The distribution of bark beetle *Ips amitinus* was determined in Czech Republic and Poland and within individual host trees (spruce). It was detected in more than half of the studied localities, from the lowland through medium upland locations to the highest localities in Tatra Mts. region. A similar pattern of *I. amitinus* occurrence from lowlands to mountains can be expected throughout Central Europe, wherever its major host (spruce) is grown. *I. amitinus* was commonly found in the middle parts of the spruce crown. When *I. typographus* abundance was high, *I. amitinus* abundance was low. The development pattern of *I. amitinus* could be very similar to that of *I. typographus*. *I. amitinus* has one generation per year in mountainous areas of Central Europe but might have two generations per year below 600 m a.s.l.

Key words: occurrence, distribution on tree, generation, Central Europe

Introduction

The bark beetle *Ips amitinus* (Eichhoff, 1872) belongs to a group of secondary insect pests of *Picea abies* (L., 1753) Karsten, 1881 that includes the bark beetles *Ips typographus* (L., 1758) and *Pityogenes chalcographus* (L., 1761) (Pfeffer, Skuhravý, 1995; Grodzki 1997, Grodzki 2004, Mazur *et al.* 2006).

I. amitinus is widespread throughout Europe where *P. abies* and *P. sylvestris* occur, but it is mostly absent in the Nordic countries (EPPO/CABI, 1997). *I. amitinus* is known from Austria, Belgium, Bulgaria, Croatia, Czech Republic, Western France, Germany, Hungary, Northern Italy, Lithuania, Latvia, FYRMacedonia, the Netherlands, Poland, Romania, Kaliningrad region, Slovakia, Slovenia, Spain (although not recently), Switzerland, Southwestern Ukraine, and Former Yugoslavia (Pavlovskij 1955, Pfeffer 1955, Jurc, Bojović 2004, Voolma *et al.* 2004). This species has expanded northward into the eastern part of Fennoscandia (Mandelshtam 1999, Koponen 1975, Annila, Nuorteva 1977, Biermann, Thalenhorst, 1977, Heliövaara *et al.* 1991, Jakovlev, Siitonen 2005). Recently, *I. amitinus* was recorded at Murmansk, north of the Arctic Circle (Voolma *et al.* 2004).

Occurrence of *I. amitinus* have been recorded on *P. abies*, *Pinus cembra* (L., 1753) (Hellrigl 1985, Stauffer, Zuber 1998), *Pinus mugo* (Turra, 1764), *Abies alba* (Miller, 1768), *Larix decidua* (Miller, 1768) (EPPO/CABI, 1997), *Pinus sylvestris* (L., 1753), and other species of *Pinus* (Knížek, Trýzna 2002, Dominik 2003). *I. amitinus* is also an important component of the fauna of blue spruce (*Picea* *pungens* (Engelmann, 1879) in Central Europe (Kula *et al.* 2009, 2011, Kula, Ząbecki 2010, Pop *et al.*, 2010)).

It often remains undetected because it is confused with other, more common species of bark beetles with which it often co-occurs (Knížek, 2001). Therefore, the occurrence and abundance of this species is not sufficiently recognized. Quality data have been provided by only a few papers, and these papers focused mainly on other species of bark beetles (Zumr 1984, Knížek 2001, Mihalciuc *et al.* 2001, Novotný *et al.* 2002, Jurc, Bojović 2004, Kuś, Kuś 2004, Grodzki *et al.* 2006, Kula *et al.* 2007, Økland, Skarpaas 2008, Grodzki 2009).

Compared with *I. typographus*, which has the same hosts as *I. amitinus* (EPPO/CABI, 1997) but is recognized as a major pest, *I. amitinus* is scarcely mentioned in the scientific literature. While the biology and ecology of *I. typographus* have been described and modelled (Lieutier 2002, Grégoire, Evans 2004, Wermelinger 2004), only sporadic information is available regarding the basic ecological characteristics of *I. amitinus* (Zumr 1982, Zuber 1992, Coeln *et al.* 1996).

The objective of this work was to determine the distribution of *I. amitinus* in an area ranging in elevation from 250-1300 m a.s.l.

Materials and Methods

Characteristics of study areas

Research was conducted in Czech Republic (one study region) and in Poland (three study regions). The research in Czech Republic was conducted in three biogeographically different areas in the eastern part of the country. The terrain of the lowest altitudes has a flat, upland character with a typical elevation of 220-300 m a.s.l. The climate is moderately warm and provides abundant precipitation (700-900 mm/y). The area has low forest cover (9%, about 30% of which is represented by spruce), with isolated forest complexes in an agricultural landscape. The terrain in the middle altitudes consists of plateaux cut by a network of deep valleys. The elevation ranges from 300 to 700 m. The climate is cold, with rainfall at 600-800 mm/y. Forest cover is 40%, of which 67% is represented by spruce. The terrain of the highest localities in Western Carpathians is characterized by mountains with elevations of 500-1,200 m. The climate is mostly cold and provides abundant rainfall. Forest cover exceeds 70%, of which spruce represents more than 70% (Culek 1996). In Poland, the research was done in three main Carpathian regions with relatively cold climates: Bieszczady Mts. study region (800-850 m, rainfall 1000-1200 mm), Gorce Mts. study region (1000-1200 m, rainfall 1000 mm), and Tatra Mts. study region (1100 m, rainfall 1100-2000 mm). In Bieszczady Mts., spruce stands consist of small islands of Norway spruce that are surrounded by beech and fir stands. Forest stands in Gorce and Tatra Mts. are dominated by spruce.

Study of trap trees for detection of *I. amitinus* at Havířov, Libavá, and Smrk Mt. in Czech Republic)

Trap trees were used in three study areas in Czech Republic: Havířov (three large forest sites: 49°49'N 18°22'E; 49°48'N 18°24'E; 49°50'N 18°22'E; indicated by small stars in Fig. 1), the area surrounding the town of Libavá (at three sites: 49°40'N 17°38'E; 49°38'N 17°30'E; 49°44'N 17°37'E; indicated by medium stars in Fig. 1), and Smrk Mt. (at one site: 49°30'N 18°22'E; indicated by the large star in Fig. 1). At each study area, spruce trees 25-35 cm in diameter (at breast height, DBH) and without beetle infestation were cut at the edge of mature forest stands (older than 80 years). The number of trap trees used at each area and in each year are indicated in Table 1. To assess bark beetle abundance, bark beetle entry holes were counted on four sections of the stem (sections were identified but the tree was left intact, i.e., it was not cut into sections). The first section (the bottom) extended from the bottom of the cut tree to 0.5 m higher on the trunk. The second section (the stem) was midway between the bottom and the beginning of the crown. The third section (the middle) was at the beginning of the crown, and the fourth section (the crown) was in the centre of the crown. On each section, one rectangular strip on the stem surface was designated; the length of the strip was equal to half of the section's circumference, and the width was 0.5-1.0 m. The circumference of each section and the strip dimensions were measured and recorded in the field.

The trap trees were debarked 6 to 7 weeks after they had been cut; this was estimated to be the time when III instar larvae and pupae of the first generation would be present. Entry holes made by bark beetles were enumerated in each strip, and the

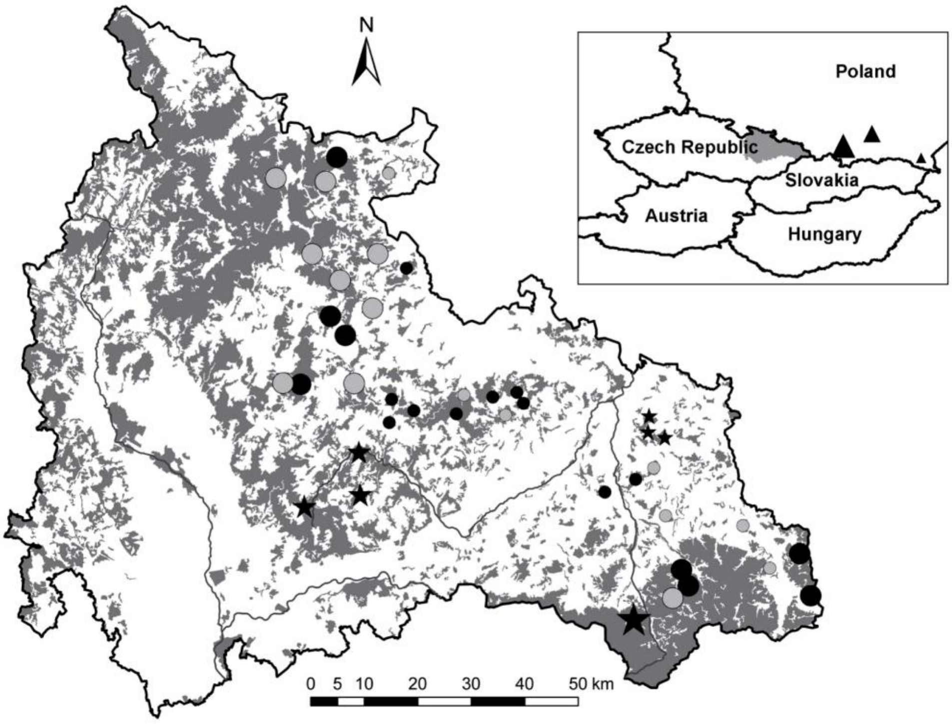

Fig. 1. Locations surveyed for occurrence of *Ips amitinus* in Czech Republic and Poland. Occurrence (presence = black symbols and absence = grey symbols) was studied using trap trees (stars), sanitary felled trees (circles), and infested trees (triangles). Small, medium, and large stars indicate Havířov, Libavá, and the Smrk Mt. (all in Czech study region), respectively. Small and large circles indicate localities lower than 500 m or higher than 500 m, respectively. The large, medium, and small triangle indicate Tatra Mts., Gorce Mts., and Bieszczady Mts. study region.

Table 1. Number of trap trees (felled in spring/summer) used at three study areas (with seven sites in total) in Czech Republic [a]. The seven sites with trap trees are indicated by stars in Fig. 1.

Study area	Altitude (m a.s.l.)	Year					
		2005	2006	2007	2008	2009	2010
Havířov	265-295	45/0	40/0	30/0	0/0	0/0	0/0
Libavá	550-655	0/0	0/0	0/0	60/52	30/16	20/20
Smrk Mt.	1180-1200	0/0	0/0	0/0	0/0	11/16	5/5

[a] Each trap tree was laid on the soil surface close to where it was cut.

species and developmental stage of the beetle(s) in the gallery associated with each entry hole were determined. The beetles were identified by examining the galleries and beetles in the field, or, if necessary, by examining the beetles after they had been taken to the laboratory.

At the study area of Havířov, trap trees were prepared in the same time period (from 15 February to 20 April) of every year (2005-2007). Entry holes were counted and bark beetles were studied on 10 June 2005, 2 June 2006, and 5 June 2007.

Traps trees were used at three sites in the area surrounding the town of Libavá and were prepared every year (2008-2010) in the same time period from 5 April to 5 May for detection of parental beetles and from 15 June to 15 July for detection of filial beetles. Entry holes were counted and bark beetles were studied on 5-8 June 2008, 15-17 August 2008, 5 June 2009, 23 July 2009, 27 June 2010, and 1 August 2010.

Trap trees were used at one site in Smrk Mt. study area and were prepared in early May and late August in 2009 and in late June and late August in 2010. Entry holes were counted and bark beetles were studied on 18 June 2009, 24 July 2009, 7 July 2010, and 20 September 2010.

Study of sanitary felled trees at 37 localities in Eastern Czech Republic

From 2006-2009, logged spruce trees were studied at 34 localities in the eastern part of Czech Republic (from 50°14’ N, 17°26’ E to 49°31’ N, 18°48’ E at elevations of 300-450 and 500-700 m; Table 2). Studied trees were between 60 and 120 years old and had been logged because they were suffering from drought, attack by *Armillaria* sp., and recent invasion by large numbers of bark beetles. The localities were visited twice yearly (May–June and August–September), and more than 10 trees were studied on each visit to each locality. If more than one beetle entry hole per dm^2 was found on a tree in at least one of its parts (base, centre of trunk, or crown), it was considered to have been invaded by large numbers of beetles. The density of entry holes was determined for three 1-m by 10-cm areas, one area for each part of the trunk. Logged trees that were dry were not studied.

Study of infested trees in Poland

Standing, infested trees in three regions in Poland (Bieszczady Mts., Gorce Mts., and Tatra Mts.) were felled, and bark beetle entry holes were counted on four areas of the stem using the same methods that has been already described. Trees were examined twice yearly (June/July and August/September/October).

The study site in Bieszczady Mts. was at 49°02’01”N, 22°51’46”E (see small triangle in Fig. 1). The trees at this site were examined twice yearly (June/July and August/September/October) in 1998-2000. A total of 47 trees were studied.

The study site in Gorce Mts. was at 49°33’00”N; 20°15’24”E (see medium triangle in Fig. 1). The trees at this site were examined twice yearly in 1999-2002 (June/July and August/September/October). A total of 35 trees were studied.

The study site in Tatra Mts. was at 49°14’40”N; 20°05’30”E (see large triangle in Fig.1). The trees at this site were examined twice yearly in 2003, 2004 and 2006(June/July and August/September/October). At total of 45 trees were studied.

Statistical analysis

Because the data were not normally distributed, nonparametric tests (Kruskal-Wallis and Mann-Whitney) were used. STATISTICA 8.0 was used for all statistical analyses.

Results

Study of trap trees at Havířov, Libavá, and Smrk Mt.

I. amitinus was detected on trap trees in all seven study sites at these three areas though not in every season (Table 3). At the lowest elevation in the study area Havířov, it was always found in June (Table 3). In middle altitudes in Libavá, *I. amitinus* was found in June 2009, June 2010, and August 2010. *I. amitinus* was most abundant in Smrk Mt. (Table 2).

At Havířov, the number of entry holes were highest in the crown section and declined toward the bottom of the tree; the numbers of entry holes per dm^2 differed between crown and bottom sections (Kruskal-Wallis test: $z=4.97$; $p<0.00001$), stem and crown sections (Kruskal-Wallis test: $z=4.66$; $p<0.0001$), and middle and crown sections (Kruskal-Wallis test: $z=3.98$; $p<0.001$). At Libavá, the number of entry holes per dm^2 did not significantly differ between the trap areas (Kruskal-Wallis test: H (3, N=760) = 1.20; $p>0.05$). At Smrk Mt., the number of entry holes per dm^2 differed among trap areas (Kruskal-Wallis test: H (3, N=145) = 14.52; $p<0.01$), and multiple comparisons confirmed that entry holes were less abundant in bottom sections than in middle (Kruskal-Wallis test: $z=2.88145$; $p<0.05$) and crown sections (Kruskal-Wallis test: $z=2.93$; $p<0.05$).

I. typographus was found in all trap trees at all three study areas. The abundances of *I. typographus* and *I. amitinus* were not correlated in 2010 at Havířov ($r=0.02$; $p>0.10$) or in June 2010 at Smrk Mt. ($r=-0.40$; $p>0.05$). The abundances of these two species were negatively correlated at Libavá in June 2009 ($r=-0.19$; $p<0.05$) and at Smrk Mt. in June 2009 ($r=-0.61$; $p<0.00001$).

Study of sanitary felled trees at 34 localities in Eastern Czech Republic

At 300-450 m, standing trees that were weak and infested by *I. amitinus* were found in all studied periods during 2006-2010, with the exception of May-June 2009 (Table 4). *I. amitinus* was found at almost 67% of the 15 localities at this elevation (small circles in Fig. 1), and the percentage of infested trees was highest in May-June 2007 (Table 4).

At 500-700 m (large circles in Fig. 1), trees infested by *I. amitinus* were detected in July and August during 2007-2009 at more than 40% of the

Table 2. Localities in Eastern Czech Republic used for study of sanitary felled trees. The 34 localities are indicated by circles in Fig. 1.

Study localities	Altitude (m a.s.l.)	Coordinates
Dolní Datyně	290	49°45’40.227”N, 18°23’39.935”E
Paskov	290	49°42’48.828”N, 18°16’23.595”E
Pazderná	300	49°40’58.12”N, 18°26’6.79”E
Václavovice	310	49°44’21.485”N, 18°20’58.906”E
Třinec	320	49°40’36.701”N, 18°38’12.827”E
Pustá Polom	350-420	49°52’3.452”N, 18°0’22.929”E
Bystřice nad Olší	390	49°36’34.239”N, 18°42’55.427”E
Slezské Rudoltice	390	50°12’49.539”N, 17°38’16.085”E
Cvilín	400	50°3’29.363”N, 17°42’26.204”E
Jánské koupele	400	49°50’11.691”N, 17°42’2.393”E
Kajlovec	400	49°51’18.456”N, 17°53’11.738”E
Dubová	450	49°49’16.328”N, 17°45’38.756”E
Jakubčovice	450	49°49’40.062”N, 17°59’57.209”E
Kyjovice	450	49°51’0.985”N, 18°2’32.887”E
Lesní Albrechtice	450-500	49°49’21.41”N, 17°52’17.111”E
Hlubočec	450-520	49°51’21.727”N, 17°57’42.44”E
Čermná	490	49°47’51.069”N, 17°41’59.768”E
Janov	500	50°13’58.133”N, 17°29’55.015”E
Bílčice	550	49°51’27.132”N, 17°35’57.913”E
Brantice	550	50°4’39.579”N, 17°37’44.326”E
Dlouhá Stráň	550	49°57’58.327”N, 17°31’16.94”E
Horní Benešov	550	49°59’10.034”N, 17°37’44.421”E
Nové Heřminovy	550	50°1’39.082”N, 17°32’15.149”E
Široká Niva	550	50°4’1.984”N, 17°27’31.291”E
Nýdek	600	49°38’15.716”N, 18°47’22.112”E
Písek	600	49°34’8.004”N, 18°49’35.106”E
Pražmo	600	49°35’42.035”N, 18°29’15.825”E
Razová	600	49°56’11.003”N, 17°33’53.499”E
Dlouhá Voda	650	50°11’25.044”N, 17°28’29.543”E
Drakov	700	50°11’17.167”N, 17°20’40.27”E
Spálené	700	50°10’4.682”N, 17°26’54.237”E
Dětřichov	750	49°50’47.596”N, 17°24’54.72”E
Krásná	900	49°34’8.493”N, 18°30’32.821”E
Ostravice	1000	49°32’45.527”N, 18°28’17.045”E

localities; the percentage of trees infested by *I. amitinus* ranged from 0 to 28% in 2007, from 0 to 9% in 2008, and from 0 to 38% in 2009. *I. typographus* was found at all localities (Table 4).

Study of infested trees in Poland

I. amitinus was found on standing infested trees in all three regions studied in Poland from June to August in all years. It was occasionally found in October. The density of entry holes ranged from 0 to 1.2 per dm^2 (Table 5).

At the site in Bieszczady Mts., the median numbers of entry holes per dm^2 differed among sections of infested trees (Kruskal-Wallis test: $H (3, N=188) = 20.20$; $p>0.001$). Multiple comparison confirmed that entry holes were less abundant in bottom sections than in stem sections (Mann-Whitney test, $z=-3.59$; $p<0.001$), middle sections (Mann-Whitney test: $z=-2.83$, $p<0.01$), and crown sections (Mann-Whitney test: $z=-3.10$; $p<0.01$).

The median numbers of entry holes per dm^2 also differed among sections of infested trees at the

Table 3. Numbers of *Ips amitinus* entry holes/dm^2 on trap trees in study areas in Eastern Czech Republic (mean±SD).

Year	Period	Study areas (Altitudes)		
		Havířov (265-295 m a.s.l.)	Libavá (520-655 m a.s.l.)	the Smrk Mt. (1260 m a.s.l.)
2005	June	0.03±0.12	–	–
2005	August	–	–	–
2006	June	0.04±0.17	–	–
2006	August	–	–	–
2007	June	0.08±0.26	–	–
2007	August	–	–	–
2008	June	–	0	–
2008	August	–	0	–
2009	June	–	0.25±0.61	1.22±1.14
2009	August	–	0	0
2010	June	–	0.01±0.02	0.22±0.17
2010	August	–	0.03±0.07	0

Table. 4. Percentage of incidentally felled trees infested by *Ips amitinus* in an amount more than one enter hole per dm^2 in Eastern Czech Republic (mean±SD).

Year	Period	Elevation (m a.s.l.)	
		300-450	500-700
2006	May-June	1.42±3.07	–
2006	July-August	23.02±3.07	–
2007	May-June	–	–
2007	July-August	1.42±1.37	5.00±4.46
2008	May-June	–	–
2008	July-August	0.89±2.67	2.88±4.46
2009	May-June	0±2.66	–
2009	July-August	5.68±3.07	8.77±3.15

site in Tatry Mts. (Kruskal-Wallis test: H (3, N=356) = 36.63; p<0.0001). Entry holes were more abundant in crown sections than in bottom sections (Mann-Whitney test: z=-3.11; p<0.01) or in stem sections (Mann-Whitney test: z=-2.63; p<0.01).

At the site in Gorce Mts., Kruskal-Wallis test indicated significant differences in median numbers of entry holes per dm^2 among sections of trees (H (3, N=136) = 15.38; p<0.01), but these differences were not confirmed by the Mann-Whitney test. No entry holes were found in bottom and stem sections, and very few were found in middle and crown sections.

I. typographus was found on all trees at all three sites in Poland. The abundances of *I. typographus* and *I. amitinus* were negatively correlated at Tatry Mts. (r=−0.19; p<0.05). Although the relationships were not statistically significant, abundances of *I. typographus* and *I. amitinus* tended to be negatively correlated at Gorce Mts. (r=-0.51; p>0.05) and the Bieszczady Mts. (r=-0.21, p>0.05).

Discussion

The presented results demonstrate that *I. amitinus* is a common species in the studied area, which is the centre of the range for this species. It was detected in more than half of the studied localities, from the lowest location through medium upland locations to the highest study sites in Tatra and Gorce Mts. regions. A similar pattern of its occurrence from lowlands to mountains can be expected wherever spruce (the major host of *I. amitinus*) is grown in Central Europe. In the present study, the number of entry holes per dm^2 of on a trap tree usually did not exceed 0.1 at lower elevations (300–450 m) and ranged from 0 to 0.2 at middle elevations (500–700 m). At high elevations of Carpathians (1000 m), which are connected to Smrk Mt. area, the number of entry holes was greater than 1 per dm^2. This high number of enter holes is the result of a local outbreak of *I. typographus* and *I. amitinus* that occurred on about 10 ha in 2007 in Smrk Natural Reserve (unpublished data). *I. amitinus* frequently appeared together with *I. typographus* in all areas. Both species reproduce at the same time (Jurc, Bojović 2004, Økland, Skarpaas 2008). On standing trees, *I. amitinus* was less abundant in the Polish study areas than in the areas in Czech Republic. Although Zumr (1984) reported that *I. amitinus* is the most abundant bark beetle in spruce mountain forests, the proportion of bark beetles represented by *I. amitinus* vs. *I. typographus* was highly variable in the current study. When *I. typographus* abundance was high, *I. amitinus* abundance was low in the present study and in previous studies (Grodzki 1997, Kula, Ząbecki 2002). In the lower elevations, *I. duplicatus* (Sahlberg, 1836) is very abundant (Holuša *et al.* 2010) and may also compete with *I. amitinus*. *I. amitinus* is probably a common and abundant species in this region of lower altitudes because the fitness of the spruce trees in this region has been reduced by drought and *Armillaria* sp. (Holuša, Liška 2002).

I. amitinus was found on both standing trees and felled trees (see also Kuś, Kuś 2004, Grodzki *et*

Table 5. Abundance of *Ips amitinus* enter holes/dm^2 on standing infested trees in Poland (mean±SD).

Year	Period	Region (Altitudes)		
		Bieszczady (800-850 m a.s.l.)	Tatras (1100 m a.s.l.)	Gorce (1000-1200 m a.s.l.)
1998	July	0,26±0,48	–	–
1998	August	0,12±0,19	–	–
1999	June	0,35±0,55	–	0,002±0,008
1999	September	0,29±0,37	–	0,03±0,14
2000	June	0,27±0,51	–	0,07±0,22
2000	September	0	–	0
2001	June	–	–	0,02±0,08
2001	September	–	–	–
2002	June	–	–	0,04±0,16
2002	September	–	–	0
2003	June	–	0,024±0,073	–
2003	August	–	–	–
2004	June	–	0,022±0,074	–
2004	August	–	0,019±0,089	–
2006	June	–	0,014±0,079	–
2006	October	–	0,014±0,079	–

al. 2006, Kula *et al.* 2007, Witrylak, 2008). Felled trees are attractive to most pest species in the genus *Ips*, including *I. typographus* (Pfeil 1827), *I. cembrae* (Heer, 1836) (Grodzki 2010), *I. acuminatus* (Gyllenhal, 1827) (Zasev 1952), and *I. sexdentatus* (Börner, 1776) (Besceli, Ekici 1969) but not *I. duplicatus* (Sierpiński 1958). Like most other *Ips* species, *I. amitinus* most often reproduces in dying or weakened trees, but it can also contribute to tree death, especially in the case of young trees and trees in plantations (Knížek 2001, Mihalciuc *et al.* 2001, Novotný *et al.* 2002, Økland, Skarpaas 2008, Grodzki, 2009).

At all localities in the current study, *I. amitinus* entry holes were more abundant in crown sections than in other sections. In contrast to *I. typographus*, *I. amitinus* prefers to breed in tree parts with relatively small dimensions (Zumr 1984, EPPO/CABI 1997). Galleries of *I. amitinus* are most often found on younger trees (Grodzki 2009), in the upper part of weakened trees, or in large-diameter weakened trees where the galleries of *I. amitinus* overlap those of *I. typographus* (Jurc, Bojović 2004). Therefore, we conclude that *I. amitinus* prefers the middle parts of the crown from the boundaries of the green branches for reproduction. This finding is consistent with previous reports (Zumr 1984, Grodzki 1997, Jakuš 1998, Kula, Ząbecki 2001, Plašil, Cudlín 2005).

On standing, infested trees at elevations up to 400 m, *I. aminitus* was abundant during two periods of the year (June–July and August–October). During both periods, we found larvae, pupae, and callow beetles in the galleries, which indicates that the beetle completes two generations per year. Unfortunately, we could not confirm this with data from trap trees because only the parental generation of bark beetles was studied on trap trees at lower elevations (Table 1). If *I. amitinus* has two generations per year at lower elevations, its development pattern would be very similar to that of *I. typographus* (Wermelinger 2004) and *I. duplicatus* (Holuљa *et al.* 2003).

Although traps trees were felled in spring and summer at medium elevations of 500–600 m, the occurrence of multiple generations per year at these elevations is unclear because there were few temporal replicates within the same site and year. The data from medium elevations are consistent with the occurrence of only one generation per year, except perhaps in Libavá in 2010, when *I. amitinus* was found in trap sections in both June and August; the abundances, however, were low.

At high elevations, a peak in detection of *I. amitinus* occurred only in June. The present study therefore confirms that *I. amitinus* completes one generation per year in the mountains. In Czech Republic, trap trees prepared in July were not infested in 2009 or 2010, although callow beetles of the first generation were found on trap trees in June and July; the data therefore indicate that the failure to detect a second generation did not result from a lack of first-generation beetles. Results from Poland also failed to indicate the development of a second generation at higher elevations (above 800 m). Although galleries of *I. amitinus* were found in August in Poland, these beetles were probably of the first and only generation. The time interval after examinations in June was only 6 weeks, and galleries detected in August likely resulted from the development of some individuals whose eggs were laid later. In September and October, galleries of *I. amitinus* were found only in a few cases. These could have been galleries produced by beetles emerging later or by sister brood. Compared with data from trap trees (which were known to be free of beetles when they were cut), data from standing trees do not provide clear information concerning time of infestation.

I. amitinus was common from the lowest locations to the highest ones in of the Carpathians in Central Europe. Its abundance was highly variable. While previous bionomic data have come only from higher elevations, where one generation of this species regularly occurs each year (Zuber 1992, Grodzki *et al.* 2008, Turčáni, Hlásny 2007, Witrylak 2008, present study), we suggest in accordance with Jurc, Bojović (2004) that at lower elevations this species will follow the pattern of *I. typographus* (Wermelinger 2004). In other words, *I. amitinus* might have two generations at elevations up to 400 m a.s.l. A second generation seems to be rare at elevations over 600 m (or over 800 m according to Zuber (1992)).

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References

Annila E., M. Nuorteva 1977. Dates of attack and emergence of *Ips amitinus* Eichh. (Col., Scolytidae) in Finland. – *Annales Entomologici Fennici*, **43**: 28-30.

Besceli O., M. Ekici 1969. Control and biology of *Ips sexdentatus* in the *Picea orientalis* region. – *Ormancilik Arastirma Enstitusu, Teknik Bulten*, **32**: 1- 32.

Biermann G., W. Thalenhorst 1977. Zur Kenntnis des kleinen Buchdruckers, *Ips amitinus* (Eichh.) (Col., Scolytidae). – *Anzeiger für Schädlingskunde Pflanzenschutz, Umweltschutz*, **50**: 20-23.

Coeln M., M. Yanzhang and E. Führer 1996. Entwicklung von Fichtenborkenkäfern in Abhängigkeit von thermischen Bedingungen verschiedener montaner Waldstufen (Coleoptera: Scolytidae). – *Entomologia Generalis*, **21**: 37-54.

Culek M. 1996. Biogeografické členění České republiky. – Enigma, Praha. 342 p.

Dominik J. 2003. Research on damage of exotic coniferous species caused by indigenous insects – supplementary results. – *Sylwan*, **10**: 25-28.

EPPO/CABI 1997. Qurantine Pests for Europe. – In: Smith I.M., D. G. McNamara, P. R. Scott, M. Holderness and B. Burger (Eds.): Data sheets on quarantine pests for the European Union and for the European and Mediterranean Plant Protection Organization.- Second Edition (eds., CAB International & European and Mediterranean Plant Protection Organization (EPPO), Wallingford, UK. 1425 p.

Grégoire J. C., H. F. Evans 2004. Damage and control of BAWBILT organisms, an overview. – In: Lieutier F., K. R. Day, A. Battisti, J. C. Grégoire and H.F. Evans (Eds.): Bark and wood boring insects in living trees in Europe, a synthesis Kluwer Academic Publishers, Dordrecht, 19-37.

Grodzki W. 1997. Changes in the occurrence of bark beetles on Norway spruce in a forest decline area in the Sudety Mountains in Poland. – In: Grégoire J. C., A.M. Liebhold, F.M. Stephen, K.R. Day and S.M. Salom (Eds.): Proceedings: Integrating cultural tactics into the management of bark beetle and reforestation pests, USDA, Forest Service General Technical Report, NE-236: 105-111.

Grodzki W. 2004. Zagrożenie górskich drzewostanów świerkowych w zachodniej części Beskidów ze strony szkodników owadzich. – *Lesne prace badawcze*, **2**: 35-47.

Grodzki W., J. Loch and P. Armatys 2006. Występowanie kornika drukarza *Ips typographus* L. w uszkodzonych przez wiatr drzewostanach świerkowych masywu Kudłonia w Gorczańskim Parku Narodowym. – *Ochrona Beskidów Zachodnich*, **1**: 125-137.

Grodzki W., M. Kosibowicz and T. Maczka 2008. Skuteczność wystawiania pułapek feromonowych na kornika drukarza *Ips typographus* (L.) w sąsiedztwie wiatrowałów i wiatrołomów. *Lesne prace badawcze*, **69**: 365-370.

Grodzki W. 2009. Entomofauna of dying young spruces *Picea abies* (L.) Karst. in the area after forest decline in the Izerskie Mountains. – *Folia Forestalia Polonica*, **51**: 161-170.

Grodzki W. 2010. Possibility of the reduction of *Ips cembrae* (Heer) (Coleoptera, Curculionidae, Scolytinae) populations in larch stands in southern Poland. – *Sylwan*, **154**: 160-167.

Heliövaara K. R. Väisänen and A. Immonen 1991. Quantitative biogeography of bark beetles (Coleoptera, Scolytidae) in Northern Europe. – *Acta Forestalia Fennica*, **219**: 35 p.

Hellrigl K. 1985. Über Borkenkäfer (Col., Scolytidae) in Zweigen der Zirbe (*Pinus cembra*) in Sudtirol. – *Anzeiger für Schädlingskunde Pflanzenschutz, Umweltschutz*, **58**: 108-110.

Holuša J., J. Liška 2002. Hypotéza chřadnutí a odumírání smrkových porostů ve Slezsku (Česká republika). – *Zprávy Lesnického výzkumu*, **47**: 9-15.

Holuša J., P. Zahradník, M. Knížek and K. Drápela 2003. Seasonal flight activity of the double-spined spruce bark-beetle *Ips duplicatus* (Coleoptera, Curculionidae, Scolytinae) in Silesia (Czech Republic). – *Biologia*, **58**: 935-941.

Holuša J., J. Lubojacký and M. Knížek 2010. Distribution of double-spined spruce bark beetle *Ips duplicatus* in the Czech Republic: spreading in 1997–2009. – *Phytoparasitica*, **38**: 435-443.

Jakovlev J., J. Siitonen 2005. Assessment of pest risk involved in timber import from northwestern Russia. – In: Health status of forests in Finland and Russian Federation (Eds.): J. Rautapää and A. Pouttu), Seminar 2005. 48 p.

Jakuš R. 1998. Patch level variation on bark beetle attack (Col., Scolytidae) on snapped and uprooted trees in Norway spruce primeval natural forest in endemic conditions: effects of host and insolation. – *Journal of Applied Entomology*, **122**: 409-421.

Jurc M., S. Bojović 2004. Bark beetle outbreaks during the last decade with special regard to the eight-toothed bark beetle (*Ips amitinus* Eichh.) outbreak in the Alpine region of Slovenia. – In: Csóka, G., A. Hirka and A. Koltay (Eds.): Biotic damage in forests. Proceedings of the IUFRO (WP7.03.10). Symposium held in Mátrafüred, Hungary, September 12-16, 2004: 85-95.

Knížek M. 2001. Progradation of small 'spruce bark beetle species'. – *Journal of Forest Science*, **47**: 113-114.

Knížek M., M. Trýzna 2002. Kůrovci na borovici vejmutovce v Národním parku České Švýcarsko. – *Zpravodaj Ochrany Lesa*, **8**: 54-55.

Koponen M. 1975. Distribution of *Ips amitinus* in Finland in 1950-1973. – *Annales Entomologici Fennici*, **41**: 65-69.

Kula E., W. Ząbecki 2001. Attractiveness of spruce for cambioxylophages as related to stand age. – *Journal of Forest Science*, **47**: 88-96.

Kula E., J. Ząbecki 2002. Struktura kambioxylofágní fauny smrku rezervace Kněhyně a hospodářských smrkových porostů v Beskydech. – *Časopis Slezského Muzea Opava* (A), **51**: 155-164.

Kula E., R. Kajfosz and W. Ząbecki 2007. Cambioxylophagous fauna of young spruce stands damaged by snow in the Beskids. – *Journal of Forest Science*, **53**: 413-423.

Kula E., R. Kajfosz and J. Polívka 2009. Cambioxylophagous fauna of blue spruce (*Picea pungens* Engelmann) in the Krušné hory Mts. (Czech Republic). – *Beskydy*, **2**: 149-156.

Kula, E., W. Ząbecki 2010. Creation of the merocoenosis of bark beetles (Scolytidae) of Norway spruce (*Picea abies* (L.) Karst.) depending on damage to trees and gradation conditions. – *Journal of Forest Science*, **56**: 474-484.

Kula E., R. Kajfosz and J. Polívka 2011. Cambioxylophagous fauna developing on logging residues of blue spruce (*Picea pungens* Engelmann). – *Journal of Forest Science*, **57**: 24-33.

Kuś, J., D. Kuś 2004. Entomofauna rozkładającego się drewna świerka, buka i brzozy na terenie Karkonoskiego Parku Narodowego. – In: Štursa, K. R. Mazurski, A. Palucki and J. Potocka (Eds.): Geoekologické problémy Krkonoš. Sborník Mezinárodní Vědecké Konference, Listopad 2003, Szklarska Poręba. – *Opera Corcontica*, **41**: 269-280.

Lieutier F. 2002. Mechanisms of resistance in conifers and bark beetle attack strategies. – In: Wagner M. R., K. M. Clancy, F. Lieutier and T. D. Paine (Eds.): Mechanisms and deployment of resistance in trees to insects. Dordrecht, Kluwer Academic Publishers, 31-77.

Mandelshtam M. J. 1999. Current status of *Ips amitinus* Eichh. (Coleoptera, Scolytidae) in North-West Russia. – *Entomologica Fennica*, **10**: 29-34.

Mazur A., A. Łabędzki and A. Raj 2006. Observations of phenology and frequencing of bark beetle (*Ips typographus* L.) and its accompanying species in the uppermost subalpine spruce forest zone environment in the Karkonoski National Park. – In: Kolk A. (Ed.): Insect outbreaks in managed and unmanaged forests Forest Research Institute, Warszawa, 29-45.

Mihalciuc V., A. Danci, D. Lupu and N. Olenici 2001. Situation of the main bark and wood boring insects which damaged conifer stands in the last 10 years in Romania. – *Anale Institutul de Cercetari si Amenjari Silvice*, **1**: 48-53.

Novotný J., W. Grodzki, M. Knížek, M. McManus and M. Turčáni 2002. The impact of spruce bark beetle populations on mountain spruce forests and ecological approaches to their management. Proceedings of the NATO Advanced Research Workshop, Stará Lesná, Slovakia, 22-26 May, 2002, IOS Press, Amsterdam, Netherlands, 250-258.

Økland B., O. Skarpaas 2008. Draft pest risk assessment report on the small spruce bark beetle, *Ips amitinus*. Commissioned report from Norwegian Forest and Landscape Institute [Oppdragsrapport fra Skog og landskap]. 20 p.

Pavlovskij E. N. 1955. Vrediteli lesa spravochnik. Izd-vo Akademii Nauk SSSR, Moskva. 1097 p.

Pfeffer A. 1955. Fauna ČSR, Kůrovci – Scolytoidea. Nakladatelství ČAV, Praha. 344 p.

Pfeffer A., V. Skuhravý 1995. Der Buchdrucker (*Ips typographus* L.) (Col. Scolytidae) und seine Problematik in der Tschechischen Republik. – *Anzeiger der Schadlingskunde, Pflanzenschutz, Umweltschutz*, **68**: 151-152.

Pfeil W. 1827. Über Insektenschaden in den Wäldern, die Mittel ihm vorzubeugen und seine Nachteile zu vermindern. Verlag Boicke, Berlin. 72 p.

Plašil P., P. Cudlín 2005. Population dynamics of eight-toothed spruce bark beetle (*Ips typographus* [L.]) in the area of National Nature Reserve Praděd in 1998–2001. – *Journal of Forest Science*, **51**: 359-370.

Pop M., E. Kula, P. Maňas and R. Kajfosz 2010. Chemical thinning in blue spruce (*Picea pungens* Engelm.) stands and its effects on cambioxylophagous fauna. – *Journal of Forest Science*, **56**: 225-235.

Sierpiński Z. 1958. Zagadnienie zwalczania kornika zrosłozębnego (*Ips duplicatus* Sahlb.). *Sylwan*, **102**: 68-75.

Stauffer C., M. Zuber 1998. *Ips amitinus* var. *montana* (Coleoptera, Scolytidae) is synonymous to *Ips amitinus*: a morphological, behavioural and genetic re-examination. – *Biochemical Systematics and Ecology*, **26**: 171-183.

Turčáni M., T. Hlásny 2007. Spatial distribution of four spruce bark beetles in north-western Slovakia. *Journal of Forest Science*, **53**: 45-52.

Voolma K., M.J. Mandelshtam, A.N. Shcherbakov, E.B. Yakovlev, H. Ounap, I. Süda, B. G. Popovichev, T. V. Sharapa, T. V. Galasjeva, R. R. Khairetdinov, V. A. Lipatkin, and E. G. Mozolevskaya 2004. Distribution and spread of bark beetles (Coleoptera: Scolytidae) around the Gulf of Finland: a comparative study with notes on rare species of Estonia, Finland and North-Western Russia. – *Entomologica Fennica*, **15**: 198-210.

WERMELINGER B. 2004. Ecology and management of the spruce bark beetle *Ips typographus* – a review of recent research. – *Forest Ecology and Management*, **202**: 67-82.

WITRYLAK M. 2008. Studies of the biology, ecology, phenology, and economic importace of *Ips amitinus* (Eichh.) (Col., Scolytidae) in experimental forests of Krynica (Beskid Sadecki, Southern Poland). – *Acta Scientiarum Polonorum Silvarum Colendarum Ratio et Industria Lignaria*, **7**: 75-92.

ZASEV B. 1952. Merki za borba srestu varhovija korojad (*Ips acuminatus* Gyll.). – *Nauchnye Trudy lesotehnitscheskogo Fakulteta selskostopanskoy Akademii, Sofia*, **1**: 109-120.

ZUBER M. 1992. Untersuchung zur Ökologie und zur Rassendifferezierung von *Ips amitinus* und *Ips amitinus* var. montana (Col., Scolytidae). PhD Thesis. ETH Zürich: Switzerland. Pages.

ZUMR V. 1982. The data for the prognosis of spring swarming of main species of bark beetles (Coleoptera, Scolytidae) on spruce (*Picea excelsea* L.). – *Zeitschrift für Angewandte Entomologie*, **93**: 305-320.

ZUMR V. 1984. Spatial distribution of bark beetles (Coleoptera, Scolytidae) in Norway spruce (*Picea excelsa* Link) and their indifference in relation to forest belts. – *Lesnictví*, **30**: 509-523.

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4.3 Pathogens of *Ips amitinus*: new species and comparison with *Ips typographus*

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Abstract

Ips amitinus and *I. typographus* are two serious pests of spruce in Europe, have similar bionomics and are likely to occur and meet on the same host trees. We therefore hypothesized that the two species support similar levels of similar pathogens. To test this hypothesis, we collected mature beetles from three trap trees at each of eight study sites and determined beetle numbers and pathogen infection levels. In total, 938 mature *I. amitinus* beetles and 3435 of *I. typographus* were dissected; five pathogens, as well as intestinal nematodes and endoparasitoids, were detected. The neogregarine *Mattesia schwenkei* is reported here for the first time as a new pathogen in 9.4% of *I. amitinus* individuals at one site. Average infection levels of most pathogens (*Chytridiopsis typographi*, *Gregarina typographi*, *Mattesia schwenkei* and parasitoids) were significantly higher in *I. typographus* than in *I. amitinus*. *Metschnikowia typographi* was confirmed only in *Ips amitinus,* while the microsporidium of *Nosema typographi* occurred only in *I. typographus*. Within-season increases in *G. typographi* infection levels were documented in *Ips amitinus*.

Introduction

The bark beetles *Ips typographus* (Linnaeus, 1758) and *Ips amitinus* (Eichhoff, 1872) are among a group of secondary insect pests of *Picea abies* [(Linnaeus, 1753) Karsten, 1881] along with *Pityogenes chalcographus* (Linnaeus, 1761) and other species (EPPO/CABI 1997; Grodzki 2004; Mazur et al. 2006). While the biology of *I. typographus* has been studied in detail (Grégoire and Evans 2004; Wermelinger 2004), relatively little information is available concerning the biology of *I. amitinus* (Zumr 1982; Coeln et al. 1996; Holuša et al. 2012).

Ips typographus and *I. amitinus* have similar tree hosts and similar interactions with their hosts (EPPO/CABI 1997). Norway spruce is the main host of both species in Europe (Pfeffer 1955; Christiansen and Bakke 1988; Voolma et al. 2004; Wermelinger 2004), although they occasionally will breed in species of *Pinus* or *Abies* and other coniferous tree species. Galleries of *I. amitinus* have also been often recorded in *Larix decidua* (Miller, 1768) (EPPO/CABI 1997). The spatial distributions of these species on the trunk often overlaps, although *I. amitinus* generally occurs in higher parts of the tree than *I. typographus* (Zumr 1984; Kula and Ząbecki 2001; Holuša et al. 2012) or prefers thinner bark for its development. At locations where the two species occur in common, they reproduce at approximately the same time (Økland and Skarpaas 2008). The proportions of the two species vary widely. When population density of one species is high, the other's abundance is lower (Kula and Ząbecki 2002).

The two species have very similar bionomics (EPPO/CABI 1997; Økland and Skarpaas 2008). After the first warm days late in May or early in June and at higher elevations, mature beetles leave their wintering refuge in the litter or under the bark of host trees (EPPO/CABI 1997; Wermelinger 2004). The males of the parental generation locate suitable stressed and dying trees where they then produce aggregation pheromones that attract both sexes to facilitate reproduction and overwhelm tree resistance (Francke et al. 1980; Wermelinger 2004). Both species are polygamous; the male fertilizes several females for which it creates a so-called nuptial chamber where reproduction occurs. Each female then burrows a maternal gallery that extends from the nuptial chamber and lays eggs along both of its sides (Wermelinger 2004). At lower elevations and depending on temperature, one or two filial generations occur during the year, with the main peaks of bark beetle emergence in July and August or September. 'Sister broods' may occur. The term 'sister brood' refers to parent beetles that re-emerge after successful reproduction and form galleries at other sites (EPPO/CABI 1997; Wermelinger 2004; Holuša et al. 2012).

In recent decades, pathogens of *I. typographus* have been intensively studied (Wegensteiner 2004; Takov et al. 2010; Wegensteiner et al. 2010; Lukášová and Holuša 2011). Diseases of *I. amitinus*, however, have been only marginally studied as part of extensive studies that mapped the pathogens of several species of bark beetles; far fewer than 2,000 individuals of *I. amitinus* have been examined in research concerning pathogens (Purrini 1978; Haidler 1998; Händel et al. 2001, 2003). In Europe, four protozoan, four microsporidia and one viral pathogen of the genus *Ips* have been determined (Wegensteiner 2004; Weiser et al. 2006; Takov et al. 2007; Holuša et al. 2009; Takov et al. 2010). The only known species-specific pathogen is the microsporidium *Larssoniella duplicati* (Weiser et al. 2006) (Fungi, Microsporidia), which occurs in the intestinal muscles of *Ips duplicatus* (Sahlberg, 1836) (Holuša et al. 2007, 2009).

As noted, previous research has shown that the two species of bark beetles have similar habitat requirements, similar bionomics and a similar spectrum of pathogens. Because they can encounter each other and one another's faeces and organic remains in galleries, especially during maturation and feeding, we presume that they may regularly transmit pathogens to one another. In this research, we tested the hypothesis that, at locations where they occur together, *I. typographus* and *I. amitinus* maintain similar infection levels of pathogens.

Materials and Methods

Ips typographus and *I. amitinus* were studied at eight sites in the Czech Republic (table 1). At each site, one sample was always collected on one date per year after the 3rd instar larvae had occurred, with the exception of the Smrk Mt. study site, where two samples were collected in 2009 and four in 2010. It is very important to document infection level of different populations in the same stage. There are two processes influenced infection level (i) incubation time of pathogens (see e.g. Wegensteiner and Weiser 2004) and (ii) pathogen transmission occurred among all members of the gallery system (Lukášová and Holuša 2011).

In late April or early May, three horizontally lying trap trees (*Picea abies*; 80–100 years old; diameter at breast height = 30–40 cm; height = 30 m) were placed approximately 30 m apart at each site. From each trap tree, beetles were collected from four sections (0.5 m long and about 0.5 m wide; the width was equivalent to half the tree circumference) located 0.5 m from the base of the trunk, halfway between the trunk base and the bottom of the crown, at the bottom of the crown and halfway between the bottom and top of the crown. For each section, the numbers of entry holes and the number of beetles of the two species were counted (mean number of entry holes and differences in attack density ±SD in table 1). Only

Table 1 Description of study sites and numbers of bark beetles during 2009–2011 in the Czech Republic

Study sites	Coordinates	Altitude (a.s.l.)	Time period	Year	n	N_{IT}	ρ_{IT} ± SD	N_{IA}	ρ_{IA} ± SD
Nová Pec	48°47′04″N,13°57′04″E	750	08 July	2009	1	71	79.3 ± 35.1	32	23.1 ± 16.9
Prášily	49°06′25″N,13°22′16″E	900	09 July	2009	1	114	69.4 ± 31.9	30	13.2 ± 11.8
Smrk Mt.	49°30′17″N,18°22′40″E	1260	26 May–18 June	2009	2	1,244	120.7 ± 79.2	250	112.7 ± 112.2
Staré Oldřůvky	49°43′01″N,17°36′32″E	550	06 June	2009	1	285	23.0 ± 30.8	61	10.7 ± 40.2
Horní Maršov	50°40′10″N,15°48′48″E	550	30 June	2010	1	101	60.6 ± 52.3	38	10.5 ± 8.1
Kozlov	49°37′58″N,17°30′16″E	650	01 August	2010	1	185	21.2 ± 32.7	30	1.5 ± 9.3
Pec pod Sněžkou	50°42′10″N,15°43′48″E	800	30 July	2010	1	394	87.5 ± 45.3	31	10.9 ± 8.6
Smrk Mt.	49°30′17″N,18°22′40″E	1260	10 June–07 July	2010	4	822	60.9 ± 35.9	436	55.9 ± 27.9
Pustá Polom	49°52′03″N,18°00′23″E	400	28 April–02 June	2011	1	219	52.7 ± 25.1	30	38.0 ± 24.8

n, number of samples; *N*, number of mature beetles (per site per sampling period); ρ, population density (number of entry holes per m^2 ± standard deviation); IT, *Ips typographus*; IA, *Ips amitinus*.

maternal beetles were collected from the individual galleries in the trap trees. The two species always occurred together in these trap trees. The individual beetles were placed in 2-cm^3 Eppendorf micro-test tubes, and a piece of wet gauze was added to maintain 100% relative humidity. The beetles were immediately frozen and stored at −4°C.

All internal organs, including the fat body, were dissected in a water drop using surgical tweezers. Each dissected beetle was examined with an Arsenal LPE 5013i-T light microscope (Arsenal s.r.o., Prague, Czech Republic) at 40–400 × magnification to determine its sex, presence of parasitoids [together *Tomicobia seitneri* (Ruschka, 1924) and *Rhopalophorus clavicornis* (Wesmael, 1835)], pathogens (in case of nematode only individuals in intestines) and the pathogen species.

The STATISTICA 9 software program was used for statistical analyses. Infection level was calculated as the percentage of beetles with positive infection at the given site (if > 30 mature beetles had been collected at the site, table 1) and mean from different sampling dates (study sites Smrk Mt. and Pustá Polom). For comparison of infection levels by pathogens, study sites with positive detection of a pathogen in at least one species were always analysed. Infection levels from all sites were compared by beetle species and genders (*t*-test or Wilcox test). At the Smrk Mt. site, the differences among collection times were tested using the Kruskal–Wallis test. Relationships between the number of entry holes of *I. typographus* or *I. amitinus* and infection levels of pathogens with more than one positive sample at the study sites were performed by regression analyses.

Results

Totals of 938 *I. amitinus* and 3,435 *I. typographus* beetles were dissected. Numbers of entry holes per m^2 (which was used as an indicator of population density) ranged from 21.2 and 120.7 for *I. typographus* and from 1.5 and 112.7 for *I. amitinus*. Average numbers of beetles were higher for *I. typographus* than for *I. amitinus* at all sites (table 1).

Both species of bark beetles contained the pathogens *Gregarina typographi* (Fuchs, 1915), *Mattesia schwenkei* (Purrini, 1970) (fig. 1), *Chytridiopsis typographi* [(Weiser, 1954) Weiser, 1970], *Ips typographus Entomopoxvirus (ItEPV)*; intestinal nematodes and eggs; and larvae of endoparasitoids (table 2). *Metschnikowia typographi* (Weiser et al. 2003) was detected in 0.1% of the individuals of *Ips amitinus* at one study site (Smrk Mt.), and the microsporidium *Nosema typographi* (Weiser, 1955) only occurred in 0.5% of the *I. typographus* beetles at one study site (table 2).

If pathogens were detected in both beetle species, we compared infection levels between the two species of bark beetles. Infection levels of intestinal nematodes were normally distributed (Shapiro–Wilk test for normality: $W = 0.96$, $P > 0.05$) and did not significantly differ between the two species (*t*-test: $t = 0.94$, $P > 0.05$); the percentage of mature beetles with nematodes in their intestines was 45.0% for *I. typographus* and 54.4% for *I. amitinus*. Infection by the *ItEPV* virus was also similar between species (Shapiro–Wilk test for normality: $W = 0.33$, $P < 0.05$; Wilcox test: $z = 1.10$, $P > 0.05$). For all other pathogens, infection levels were significantly higher in mature *I. typographus* than in mature *I. amitinus*. The microsporidium *C. typographi* was always more abundant in *I. typographus* (mean 4.8%) than in *I. amitinus* (mean 0.6%) at all seven study sites where it was detected (Shapiro–Wilk test for normality: $W = 0.50$, $P < 0.00001$; Wilcox test: $z = 2.37$, $P < 0.05$; fig. 2). Infection level of *C. typographi* at the study site Pustá Polom was very low and, moreover, confirmed only in *I. typographus*. No differences were recorded between sampling dates.

The protozoan pathogen *G. typographi* was detected at four study sites. Its infection level was not normally distributed (Shapiro–Wilk test for normality: $W = 0.50$, $P < 0.00001$), and significant differences were detected between the species (Wilcox test: $z = 2.52$, $P < 0.05$; fig. 2). In the case of *G. typographi*,

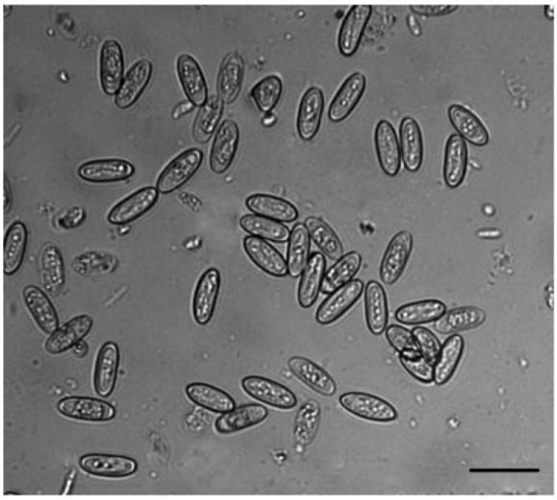

Fig. 1 Spores of *Mattesia schwenkei* in the haemolymph of *Ips amitinus*; bar = 20 μm (micrograph by K. Lukášová).

Table 2 Pathogen infection levels ± standard deviation at the study sites

Study site	Year	Beetle	Percentage of beetles with the indicated pathogen N_i	Ch_t	N_t	M_t	G_t	M_s	*ItEPV*	Par
Nová Pec	2009	IA	68.8 ± 5.0	–	–	–	–	9.4 ± 0.8	–	6.3 ± 3.3
Nová Pec	2009	IT	45.1 ± 16.1	1.4 ± 1.7	–	–	–	29.6 ± 14.9	5.6 ± 6.8	7.0 ± 2.4
Prášily	2009	IA	76.0 ± 13.3	–	–	–	–	–	–	–
Prášily	2009	IT	54.4 ± 4.7	4.4 ± 3.3	–	–	2.6 ± 4.1	11.4 ± 1.2	0.9 ± 0.7	10.5 ± 1.1
Smrk Mt.	2009	IA	40.4 ± 24.6	–	–	0.1 ± 0.3	15.2 ± 12.1	–	–	0.3 ± 0.9
Smrk Mt.	2009	IT	53.1 ± 28.4	0.02 ± 0.2	–	–	18.4 ± 17.4	–	–	0.2 ± 3.0
Staré Oldřůvky	2009	IA	24.6 ± 10.9	4.9 ± 10.9	–	–	–	–	–	1.6 ± 1.9
Staré Oldřůvky	2009	IT	29.8 ± 23.3	10.2 ± 10.1	–	–	–	–	–	0.4 ± 3.0
Horní Maršov	2010	IA	94.7 ± 1.5	–	–	–	5.3 ± 1.5	–	2.6 ± 3.6	–
Horní Maršov	2010	IT	90.1 ± 6.7	–	–	–	11.9 ± 1.9	–	–	3.0 ± 1.5
Kozlov	2010	IA	35.0 ± 18.4	–	–	–	–	–	–	–
Kozlov	2010	IT	14.1 ± 8.7	19.5 ± 10.3	–	–	–	–	–	1.1 ± 1.4
Pec pod Sněžkou	2010	IA	20.0 ± 16.3	–	–	–	–	–	–	–
Pec pod Sněžkou	2010	IT	21.3 ± 16.8	4.6 ± 4.2	0.5 ± 1.0	–	2.0 ± 2.1	6.9 ± 6.2	17.8 ± 7.3	1.5 ± 1.4
Smrk Mt.	2010	IA	51.0 ± 7.8	–	–	–	4.6 ± 1.4	–	–	–
Smrk Mt.	2010	IT	58.7 ± 15.2	–	–	–	19.0 ± 9.5	3.8 ± 5.5	–	1.3 ± 0.7
Pustá Polom	2011	IA	78.9 ± 23.6	–	–	–	–	–	–	–
Pustá Polom	2011	IT	38.8 ± 12.8	2.7 ± 1.7	–	–	–	–	–	–

IT, *Ips typographus*; IA, *Ips amitinus*; N_i, gut nematodes; Ch_t, *Chytridiopsis typographi*; N_t, *Nosema typographi*; M_t, *Metschnikowia typographi*; G_t, *Gregarina typographi*; M_s, *Mattesia schwenkei*; *ItEPV*, *Entomopoxvirus typographi*; Par, endoparasitoids.

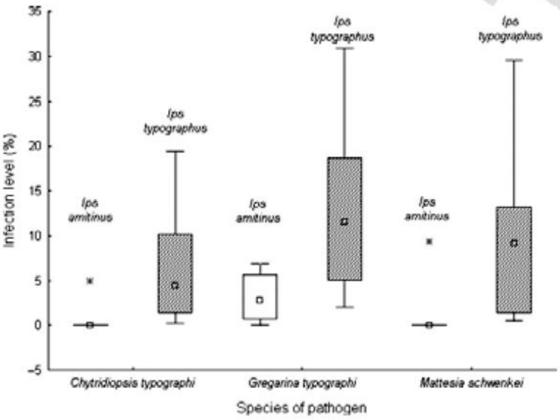

Fig. 2 Percentage of *Ips amitinus* and *Ips typographus* infected by *Chytridiopsis typographi*, *Gregarina typographi* and *Mattesia schwenkei*. Box plots show median plus upper and lower quartiles for all seven sites where the pathogen was detected. Minimum and maximum values are shown by the upper and lower whiskers (1.5 × interquartile range). Circles indicate outlying values, and stars indicate extreme values.

infection of *I. amitinus* within nuptial chambers apparently increased during the 2009 season at Smrk Mt., that is, infection was significantly higher in the last (28.2%) than in the first collection (1.5%) in 2009 (Kruskal–Wallis test: $H = 10.70$, $P < 0.01$; fig. 3). In 2010, on the other hand, infection of *I. amitinus* by *G. typographi* did not significantly differ between the individual dates (Kruskal–Wallis test: $H = 2.63$, $P > 0.05$; fig. 3).

At four study sites where the neogregarine *M. schwenkei* was detected, infection was also significantly higher in *I. typographus* than in *I. amitinus* (Shapiro–Wilk test for normality: $W = 0.49$, $P < 0.00001$; Wilcox test: $z = 2.20$, $P < 0.05$; fig. 2). At Nová Pec, infection of *I. amitinus* by *M. schwenkei* (fig. 1) was relatively high. In total, 9.4% of the mature beetles were infected (table 2).

Parasitoids (Hymenoptera: Braconidae and Pteromalidae) more frequently attacked *I. typographus* than *I. amitinus* (Shapiro–Wilk test for normality: $W = 0.62$, $P < 0.00001$; Wilcox test: $z = 2.27$, $P < 0.05$).

Differences were never found in pathogen infection rates of male and female beetles of both species. All analyses (Wilcox test) were not statistically significant for *I. amitinus* (intestinal nematodes: $z = 1.13$; *G. typographi*: $z = 0.51$; endoparasitoids: $z = 0.13$; $P > 0.2$) and *I. typographus* (intestinal nematodes: $z = 1.75$; *C. typographi*: $z = 0.42$; *G. typographi*: $z = 1.36$; *M. schwenkei*: $z = 1.12$; *ItEPV*: $z = 0.17$; endoparasitoids: $z = 1.22$; $P > 0.05$).

A conclusive linear correlation between the number of entry holes of *I. amitinus* and infection levels of pathogens was not statistically significant (intestinal nematodes: $r = -0.07$; *G. typographi*: $r = 0.39$; endoparasitoids: $r = -0.09$; in all cases: $P > 0.01$). A similar

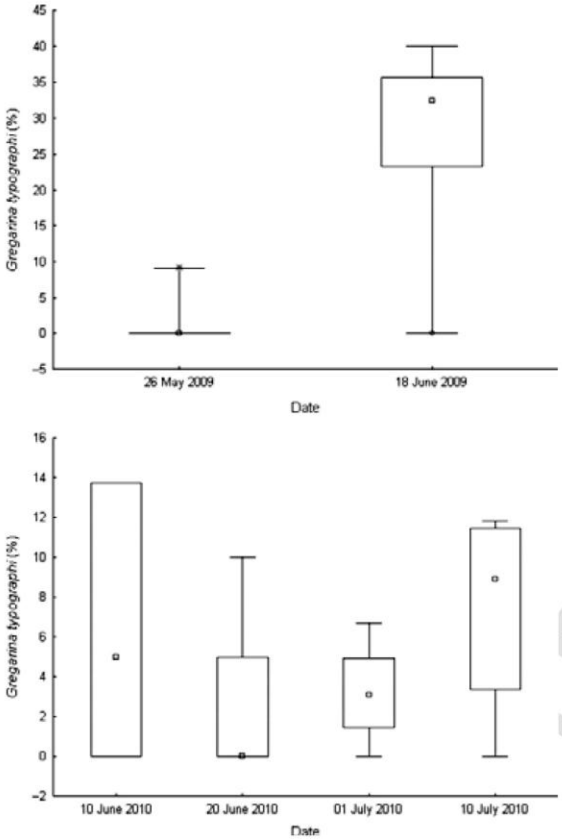

Fig. 3 Percentage of *Ips amitinus* infected by *Gregarina typographi* per trap tree in 2009 (upper graph) and 2010 (lower graph) at one study site (Smrk Mt.). Box plots show median plus upper and lower quartiles. Minimum and maximum values are shown by the upper and lower whiskers (1.5 × interquartile range). Circles indicate outlying values, and stars indicate extreme values.

result was also found between the number of entry holes of *I. typographus* and infection levels of pathogens (intestinal nematodes: $r = 0.31$; *C. typographi*: $r = -0.70$; *G. typographi*: $r = 0.50$; *M. schwenkei*: $r = 0.27$; *ItEPV*: $r = 0.35$; endoparasitoids: $r = 0.15$; in all cases: $P > 0.05$).

Discussion

To date, only 10 species of pathogens (excluding nematodes and entomopathogenic fungi) have been described in *Ips typographus* and six have been described in *I. amitinus* (Wegensteiner 2004; Takov et al. 2010; recent study). Because no study has examined even as many as 1,000 *I. amitinus* beetles, the possible existence of additional pathogens of *I. amitinus* cannot be excluded.

While the microsporidium *C. typographi* can be found in *I. typographus* and *I. amitinus*, it also occurs in other species of bark beetles (Haidler 1998; Händel et al. 2001). Therefore, it is apparently a non-specific pathogen in the intestine of a number of pests in coniferous stands (Wegensteiner 2004). The infection level of this pathogen varies greatly (Wegensteiner and Weiser 2004; Holuša et al. 2009; Wegensteiner et al. 2010). In our study, *C. typographi* was the most common pathogen and was confirmed at seven sites, with infection levels ranging from 0.2 to 19.5%.

The microsporidium *N. typographi* was detected in the adipose tissue of only 0.5% of mature *I. typographus* beetles, that is in line with other studies, in which the infection level has always been low (Wegensteiner and Weiser 1996a; Händel et al. 2003).

With the exception of *M. typographi*, fungal pathogens other than microsporidia are uncommon in *I. typographus* (Wegensteiner 2004) and *I. amitinus* (Händel et al. 2001; Weiser et al. 2003; Unal et al. 2009). In the present study, *M. typographi* was detected only in *I. amitinus* at only one site and at a low level of infection.

The only known virus in the midgut epithelium affecting the genus *Ips* is *ItEPV*, and it has been detected at a number of locations (Weiser and Wegensteiner 1994; Wegensteiner and Weiser 1995; Haidler 1998; Weiser et al. 2000; Händel et al. 2001; Burjanadze and Goginashvili 2009; Yaman and Baki 2011). In the present work, *ItEPV* infection levels did not significantly differ between *I. typographus* and *I. amitinus*, perhaps because the virus was detected in only a few sites. The analysis should be repeated with a larger number of sites.

Gregarina typographi occurs in the midgut of a number of representatives of the Scolytinae subfamily, and its infection level varies greatly among individual sites (Takov et al. 2007; Yaman 2007; Holuša et al. 2009; Kereselidze et al. 2010; Takov et al. 2010). *G. typographi* is currently not considered a lethal pathogen and is characterized as having low virulence (Tanada and Kaya 1993; Yaman 2007; Wegensteiner et al. 2010).

In this study, *M. schwenkei* was detected for the first time in the fat body of *I. amitinus*. It is a common species in *I. typographus* and other bark beetles (Purrini 1978; Händel et al. 2003). *M. schwenkei* was first described from *Dryocoetes autographus* (Purrini 1977).

Most intestinal nematodes in bark beetles are parasitic species that are difficult to identify because they are usually invasive larvae, which lack distinguishing morphological characteristics, and because identification in certain groups is impossible without preservation (Rühm 1956). In the current study, intestinal nematodes were detected in about 50% of the bark beetles, an parasitization level similar to that reported in other studies (Burjanadze and Goginashvili 2009; Kereselidze et al. 2010). The effect of these nematodes on bark beetles in the *Ips* genus has been infrequently studied (Lieutier 1980; Forsse 1987), and we still have insufficient evidence regarding their effects on the beetles.

Common and well-studied endoparasitoids of adults in the *Ips* genus are the parasitic wasps *Tomicobia seitneri* and *Rhopalophorus clavicornis* (Faccoli 2000, 2001). They have similar bionomics and attack and lay eggs in mature beetles even before the beetles bore into the tree and also attack mature and feeding beetles in the galleries (Kenis et al. 2004). Because the level of attack by parasitoids depends on host density, it is reasonable that the percentage of parasitism by endoparasitoids was higher for *I. typographus* than for *I. amitinus* because the former species was more abundant.

Ips typographus is the most common species of spruce bark beetle and rapidly increases to large numbers in older spruce stands at both high and low elevations (Wermelinger 2004). In contrast, outbreaks of *I. amitinus* occur only rarely and only over small areas. *I. amitinus* is generally less abundant than *I. typographus* in forests (as it was in this study), and it occurs in higher mountain areas, where, moreover, it only completes one generation per year (Holuša et al. 2012). This can explain why, although both beetles share similar pathogens, infection levels were lower for *I. amitinus* than for *I. typographus* in the case of those pathogens that are transferred perorally by infected faeces (pathogens localized in the intestine, including *C. typographi* and *G. typographi*) and those passed on after the death of the host organism (pathogens localized in the fat body, such as *M. schwenkei*); infection levels by these pathogen species are influenced by beetle population density. If densities are low, the beetles do not encounter individuals from other galleries, pathogen spores are only transferred among beetles in one gallery system, and infection of other beetles by faeces and the remains of dead bodies are almost excluded (Wegensteiner and Weiser 1996b). This situation is typical for locations with managed forests. In unmanaged forests, bark beetles can become more abundant and concentrated, resulting in a greater probability of pathogen transmission and therefore higher levels of infection (Holuša et al. 2009). These facts are not in contrary with results of this study that found relationships between bark beetles densities and pathogens infection levels. We know nothing about duration of bark beetles outbreaks as well about pathogens of initial population.

Another factor affecting the infection rate at a given place is the time of collection during the season, because individuals inhabiting the same gallery transmit the infection among themselves and the infection level can increase substantially within a single season. This was confirmed at the Smrk Mt. site for *I. typographus* (Lukášová and Holuša 2011), where population densities of both species were very high. In 2009, a large increase in the *G. typographi* infection through the season was also evident in mature *I. amitinus* beetles. The situation at Smrk Mt. was a little different in 2010 in that *G. typographi* infection in *I. amitinus* at the end of the study was higher than in the previous collections but the difference was not statistically significant. The difference between the two species can probably be explained by a markedly lower infection level by gregarines in *I. amitinus* during 2010 at Smrk Mt., which did not vary sufficiently to result in statistical significance. At other sites, maternal beetles of both species were collected in similar development phases (i.e. the larvae of both species were already in the 3rd instar within the galleries), indicating that they had inhabited the galleries for approximately the same length of time, which facilitates the comparison of infection levels between the two species. *C. typographi* must be ingested actively by host species; up to now, such coprophagous habits were only presumed (Wegensteiner and Weiser 1996a,b) but not proved for bark beetles. This immediate availability of infectious units in the case of *C. typographi* can explain why this species can be found not only in a high proportion of parental beetles, but also (after a certain incubation period) in a relatively high number of offspring beetles. On the other hand, *G. typographi* needs a period of maturation outside their host before being ingested again (Wegensteiner and Weiser 2004).

The risk of infection with all pathogens appeared similar for both sexes of both species, which corresponds to the report by Wegensteiner et al. (1996) and Lukášová and Holuša (2011). Both sexes come in contact with contaminated faeces at the same time during breeding, egg-laying and removal of frass from entry holes. These findings show that the risk of infection is similar for both sexes and that the infection spreads more or less evenly among males and females. In contrary, some works (Wegensteiner and Weiser 2004, 1996a,b) reported infection levels of *G. typographi* or *C. typographi* higher for *I. typographus* males. They suggested that because one male is in contact with two to three females within one breeding gallery, it can explain the higher infection rates in male beetles.

In summary, *I. amitinus* hosts the same species of pathogens as *I. typographus*, although at lower infection levels. This probably results from less frequent outbreaks and lower population densities for *I. amitinus*. Low population densities reduce the probability of beetles contacting each other beneath the bark and thus reduce the probability of pathogen transmission.

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References

Burjanadze M, Goginashvili N, 2009. Occurrence of pathogens and nematodes in the spruce bark beetles, *Ips typographus* (Col., Scolytidae) in Borjomi gorge. Bull. Georgian Natl. Acad. Sci. 3, 145–149.

Christiansen E, Bakke A, 1988. The spruce bark beetle of Eurasia. In: Dynamics of forest insect populations. Ed. by Berryman AA, Plenum Press, New York, 479–503.

Coeln M, Yanzhang M, Führer E, 1996. Entwicklung von Fichtenborkenkäfern in Abhängigkeit von thermischen Bedingungen verschiedener montaner Waldstufen (Coleoptera: Scolytidae). Entomol. Gen. 21, 37–54.

EPPO/CABI, 1997. Qurantine Pests for Europe. In: Data sheets on quarantine pests for the European Union and for the European and Mediterranean Plant Protection Organization, 2nd edn. Ed. by Smith IM, McNamara DG, Scott PR, Holderness M, Burger B, CAB International & European and Mediterranean Plant Protection Organization (EPPO), Wallingford, 1425.

Faccoli M, 2000. Notes on the biology and ecology of *Tomicobia seitneri* (Ruschka) (Hymenoptera: Pteromalidae), a parasitoid of *Ips typographus* (L.) (Coleoptera: Scolytidae). Frustula Entomol. 23, 47–55.

Faccoli M, 2001. *Tomicobia seitneri* (Ruschka), *Ropalophorus clavicornis* (Wesmael) and *Coeloides bostrychorum* Giraud: three hymenopterous parasitoids of *Ips typographus* (L.) (Col., Scolytidae) new to Italy. Boll. Soc. Entomol. Ital. 133, 237–246.

Forsse E, 1987. Flight duration in *Ips typographus* L.: insensitivity to nematode infection. J. Appl. Entomol. 104, 326–328.

Francke W, Sauerwein P, Vité JP, Klimetzek D, 1980. The pheromone bouquet of *Ips amitinus*. Naturwissenschaften 67, 147.

Grégoire J-C, Evans HF, 2004. Damage and control of BAWBILT organisms, an overview. In: Bark and wood boring insects in living trees in Europe, A synthesis. Ed. by Lieutier F, Day KR, Battisti A, Grégoire J-C, Evans HF, Kluwer, Dordrecht, 19–37.

Grodzki W, 2004. Zagrozenie górskich drzewostanów swierkowych w zachodniej czésci Beskidów ze strony szkodników owadzich. Leś. Pr. Badawcze 2, 35–47.

Haidler B, 1998. Pathogene und Parasiten von Fichtenborkenkäfern in einem Fichtenbestand am Achner Kogel bei Tamsweg. Diploma thesis, Formal- und Naturwissenschaftliche Fakultät der Universität Wien, 144.

Händel U, Kenis M, Wegensteiner R, 2001. Untersuchungen zum Vorkommen von Pathogenen und Parasiten in Populationen überwinternder Fichtenborkenkäfer (Col., Scolytidae). Mitt. Dtsch. Ges. Allg. Angew. Entomol. 13, 423–428.

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. J. Pest. Sci. 76, 22–32.

Holuša J, Weiser J, Drápela K, 2007. Pathogens of *Ips duplicatus* (Coleoptera, Scolytidae) in three areas in Central Europe. Acta Protozool. 46, 157–167.

Holuša J, Weiser J, Žižka Z, 2009. Pathogens of the spruce bark beetles *Ips typographus* and *Ips duplicatus*. Cent. Eur. J. Biol. 4, 567–573.

Holuša J, Lukášová K, Grodzki W, Kula E, Matoušek P, 2012. Is *Ips amitinus* (Coleoptera: Curculionidae) abundant in wide range of altitudes? Acta Zool. Bulg. ????, ????–???? (in press).

Kenis M, Wermelinger B, Grégoire J-C 2004. Research on parasitoids and predators of Scolytidae – a review. In: Bark and wood boring insects in living trees in Europe, A synthesis. Ed. by Lieutier F, Day KR, Battisti A, Grégoire J-C, Evans HF, Kluwer, Dordrecht, 237–290.

Kereselidze M, Wegensteiner R, Goginashvili N, Tvaradze M, Pilarska D, 2010. Further studies on the occurrence of natural enemies of *Ips typographus* (Coleoptera: Curculionidae: Scolytinae) in Georgia. Acta Zool. Bulg. 62, 131–139.

Kula E, Ząbecki W, 2001. Attractiveness of spruce for cambioxylophages as related to stand age. J. For. Sci. 47, 88–96.

Kula E, Ząbecki J, 2002. Struktura kambioxylofágní fauny smrku rezervace Kněhyně a hospodářských smrkových porostů v Beskydech. Čas. Slezs. Muz. Opava (A) 51, 155–164.

Lieutier F, 1980. Le parasitisme d'*Ips sexdentatus* (Boern) (Coleoptera: Scolytidae) par les nématodes du genre *Parasitaphelenchus* Fuchs. Relations avec le parasitisme par

Contortylenchus diplogaster (v. Lins.). Rev. Nématol. 3, 271–281.

Lukášová K, Holuša J, 2011. *Gregarina typographi* (Eugregarinorida: Gregarinidae) in th bark beetle *Ips typographus* (Coleoptera: Curculionidae): changes of infection level in breeding system. Acta Protozool. 50, 311–318.

Mazur A, Łabędzki A, Raj A, 2006. Observations of phenology and frequencing of bark beetle (*Ips typographus* L.) and its accompanying species in the uppermost subalpine spruce forest zone environment in the Karkonoski National Park. In: Insect outbreaks in managed and unmanaged forests. Ed. Kolk A, Forest Research Institute, Warszawa, 29–45.

Økland B, Skarpaas O, 2008. Draft pest risk assessment report on the small spruce bark beetle, *Ips amitinus*. Commissioned report from Norwegian Forest and Landscape Institute, 10/08, 20.

Pfeffer A, 1955. Fauna ČSR, Kůrovci – Scolytoidea. Nakladatelství ČAV, Praha, 344

Purrini K, 1977. Über eine neue Schizogregarinen-Krankheit der Gattung *Mattesia* Naville (Sporozoa, Dischizae) des Zottigen Fichtenborkenkäfers, *Dryocoetes autographus* Ratz (Coleoptera, Scolytidae). Anz. Schädl., Pflanz., Umwelt. 50, 132–135.

Purrini K, 1978. Protozoen als Krankheitserreger bei einigen Borkenkäferarten (Col., Scolytidae) im Königsee-Gebiet, Oberbayern. Anz. Schädl., Pflanz., Umwelt. 51, 171–175.

Rühm W, 1956. Die Nematoden der Ipiden: Parasitologische Schriftenreihe, 6. Veb Gustav Fischer Verlag, Jena, 437.

Takov D, Doychev D, Wegensteiner R, Pilarska D, 2007. Study on the pathogens of bark beetles (Coleoptera, Scolytidae) from different coniferous stands in Bulgaria. Acta Zool. Bulg. 59, 87–96.

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 Zoolog. Bulg. 62, 201–209.

Tanada Y, Kaya HK, 1993. Protozoan infection: Apicomplexa, Microspora. In: Insect pathology. Ed. by ???? ????, Academic Press, San Diego, 414–458.

Unal S, Yaman M, Tosun O, Aydin C, 2009. Occurence of *Gregarina typographi* (Apicomplexa, Gregarinidae) and *Metschnikowia typographi* (Ascomycota, Metschnikowiaceae) in *Ips sexdentatus* (Coleoptera: Cueculionidae, Scolytinae) populations in Kastamonu (Turkey). J. Anim. Vet. Adv. 8, 2687–2691.

Voolma K, Mandelshtam MJ, Shcherbakov AN, Yakovlev EB, Ounap H, Süda I, Popovichev BG, Sharapa TV, Galasjeva TV, Khairetdinov RR, Lipatkin VA, Mozolevskaya EG, 2004. Distribution and spread of bark beetles (Coleoptera: Scolytidae) around the Gulf of Finland: a comparative study with notes on rare species of Estonia, Finland and North-Western Russia. Entomol. Fenn. 15, 198–210.

Wegensteiner R, 2004. Pathogens in bark beetles. In: Bark and wood boring insects in living trees in Europe, A synthesis. Ed. by Lieutier F, Day KR, Battisti A, Grégoire J-C, Evans HF, Kluwer, Dordrecht, 291–313.

Wegensteiner R, Weiser J, 1995. A new *Entomopoxvirus* in the bark beetle *Ips typographus* (Coleoptera: Scolytidae). J. Invertebr. Pathol. 65, 203–205.

Wegensteiner R, Weiser J, 1996a. Untersuchungen zum Auftreten von Pathogen bei *Ips typographus* (Coleoptera: Scolytidae) aus einem Naturschutzgebiet in Schwarzwald (Baden-Württemberg). Anz. Schädl., Pflanz., Umwelt. 69, 162–167.

Wegensteiner R, Weiser J, 1996b. Occurrence of *Chytridiopsis typographi* (Microspora, Chytridiopsida) in *Ips typographus* L. (Col., Scolytidae) field population and in a laboratory stock. J. Appl. Entomol. 120, 595–602.

Wegensteiner R, Weiser J, 2004. Annual variation of pathogen occurrence and pathogen prevalence in *Ips typographus* L. (Col, Scolytidae) from the BOKU University Forest Demonstration Centre. J. Pest. Sci. 77, 221–228.

Wegensteiner R, Weiser J, Führer E, 1996. Observations on the occurrence of pathogens in the bark beetle *Ips typographus* L. (Coleoptera. Scolytidae). J. Appl. Entomol. 120, 199–204.

Wegensteiner R, Dedryver C-A, Pierre J-S, 2010. The comparative prevalence and demographic impact of two pathogens in swarming *Ips typographus* matures: a quantitative analysis of long term trapping data. Agric. For. Entomol. 12, 49–57.

Weiser J, Wegensteiner R, 1994. A new *Entomopoxvirus* in the bark beetle *Ips typographus* (Coleoptera, Scolytidae) in Czechoslovakia. Z. Angew. Zool. 80, 425–434.

Weiser J, Pultar O, Žižka Z, 2000. Biological protection of forest against bark beetle outbreaks with poxvirus and other pathogens. IUAPPA Section B, 168–172.

Weiser J, Wegensteiner R, Händel U, Žižka Z, 2003. Infections with the Ascomycete *Metschnikowia typographi* n. sp. in the bark beetle *Ips typographus* and *Ips amitinus* (Col., Scolytidae.). Folia Microbiol. 48, 611–618.

Weiser J, Holuša J, Žižka Z, 2006. *Larssoniella duplicati* n.sp. (Microsporidia, Unikaryonidae) in the double – spined spruce bark beetle, *Ips duplicatus* (Coleoptera, Scolytidae) in Central Europe. J. Pest. Sci. 79, 127–135.

Wermelinger B, 2004. Ecology and management of the spruce bark beetle *Ips typographus* – a review of recent research. Forest Ecol. Manage. 202, 67–82.

Yaman M, 2007. *Gregarina typographi* Fuchs, a gregarine pathogen of the six-toothed pine bark beetle, *Ips sexdentatus* (Boerner) (Coleoptera: Curculionidae, Scolytinae) in Turkey. Turk. J. Zool. 31, 359–363.

Yaman M, Baki H, 2011. First Record of *Entomopoxvirus* of *Ips typographus* (Linnaeus) (Coleoptera: Curculionidae, Scolytinae) for Turkey. Acta Zoolog. Bulg. 63, 199–202.

Zumr V, 1982. The data for the prognosis of spring swarming of main species of bark beetles (Coleoptera, Scolytidae) on spruce (*Picea excelsea* L.). Z. Angew. Entomol. 93, 305–320.

Zumr V, 1984. Spatial distribution of bark beetles (Coleoptera, Scolytidae) in Norway spruce (*Picea excelsa* Link) and their indifference in relation to forest belts. Lesnictví 30, 509–523.

4.4 Bionomy of *Ips cembrae* (Coleoptera: Curculionidae: Scolytinae): flight activity, dispersion on trap trees and overwintering

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2012

Šumarski list, odesláno do redakce.

BIONOMY OF *IPS CEMBRAE* (COLEOPTERA: CURCULIONIDAE: SCOLYTINAE): FLIGHT ACTIVITY, DISPERSION ON TRAP TREES AND OVERWINTERING

BIONOMIJA POTKORNJAKA IPS CEMBRAE *(COLEOPTERA: CURCULIONIDAE: SCOLYTINAE): ROJENJE, DISPERZIJA NA LOVNIM STABLIMA I PREZIMLJAVANJE*

Running title: BIONOMY OF *IPS CEMBRAE*

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Abstract

The threat to forests from a nascent gradual increase of *Ips cembrae* necessitates more precise knowledge as to the bionomics, ethology, reproduction and population dynamics, monitoring and protection against this pest.

The effectiveness of trap trees and Cembräwit® pheromone traps for *Ips cembrae* was evaluated during 2007–2009 at outbreaks in the Czech Republic. From the end of September the sets of logs were gradually placed into emergence traps in laboratory conditions in monthly intervals (November–January). At the end of March, emergence traps with area of 0.5 m^2 were placed on ground to collect litter leaving beetles.

Spring emergence corresponded to the fly-out from May to mid-June and which culminated in mid-May. Trap trees were invaded continuously and evenly along the entire profile of the trunk. Parent galleries in the upper parts of a trap tree’s trunk were longer and numbers of deposited eggs were lower. Gradual fly-out of beetles from infested larch wood under laboratory conditions during the course of the winter confirmed the presence of overwintering beetles, larvae and pupae of the offspring generation. Beetles overwinter near the trees where they completed their development.

Keywords: *Ips cembrae*, pheromone-baited trap, trap tree, flight activity, dispersion, overwintering

INTRODUCTION

UVOD

Large larch bark beetle *Ips cembrae* (Heer, 1836) occurs in Europe (Austria, Croatia, Czech Republic, Denmark, Finland, France, Germany, Hungary, Great Britain, Italy, England, Wales, Scotland, Netherlands, Poland, Romania, Serbia and Montenegro, Slovenia, Slovakia, Sweden, Switzerland and Ukraine) and Central Russia (OEPP/EPPO 2005). The occurrence of *I. cembrae* was incorrectly indicated to be in eastern Asia (Postner 1974), whereas it is the species *Ips subelongatus* Motschulsky, 1860 (Stauffer al. 2001, Zhang et al. 2007) that is described in some works of (Terasaki et al. 1987, Yamaguchi et al. 1989, Zhang et al. 1992, Suzuki and Imada 1993, van der Westhuizen et al. 1995, Yamaoka et al. 1998, Zhang et al. 2000).

The main host plant for *I. cembrae* is the larch *Larix decidua* Mill. in the entire area of its distribution, from the lowest altitudes to the subalpine zone (Postner 1974). It occasionally colonizes the spruce *Picea abies* (L.) Karsten (Pfeffer 1989). Reported infestation on pine (*Pinus cembra* L.) has been revised [confusion with *Ips amitinus* (Eichhoff, 1871)] (Pfeffer 1995).

I. cembrae is a secondary pest in larch plantations (Grégoire and Evans 2004), breeding in logs (Elsner 1997), wind-blown trunks (Krehan and Steyer 2005) or storm-damaged trees (Luitjes 1974) and dying trees (Grodzki 2008). Drought conditions at drier sites may promote infestation on green trees (Bevan 1987). In such cases, it breeds and subsequently acts as a primary pest in healthy trees. Young and older stands in lower and medium altitudes are threatened (Grodzki and Kosibowicz 2009). As they increase in numbers, they can cause harm to larch stands as a physiological pest even in visually healthy, standing trees that succumb to mass invasion, and as a defoliator as a result of maturation feeding by young beetles upon thin twigs in the crowns of healthy trees or regeneration feeding of older beetles in thin trunks or thicker branches (Postner 1974, Krehan and Cech 2004).

The basic bionomics of *I. cembrae* is known, and they differ in details from the bionomics of other species of the *Ips* genus. Recently, however, there have been only a few works dealing with the bionomics of this species and protection against it. Inasmuch as in 2006 the species began gradually to increase in many places of the Czech Republic (Knížek 2008), this article aims to define:

(i) flight activity, to specify study an approach using artificial lures evaporators;

(ii) distribution in time and space on trap trees;

(iii) overwintering of beetles.

MATERIAL AND METHODS

MATERIJAL I METODE RADA

The research was carried out near the villages of Slezské Rudoltice (50°12'37.827"N, 17°38'52.579"E) (2007–2008) and Útěchov (49°17'12.646"N, 16°37'15.632"E) (2008–2009). At the Slezské Rudoltice locality, we selected a forest stand of 1.55 ha, 56 years old with close canopy, larch dominancy of 90%, average $d_{1.3}$ of 23 cm, and average height of 23 m. At the Útěchov locality, we selected a forest stand of 7.02 ha, 88 years old with close canopy, larch dominancy of 44%, average $d_{1.3}$ of 29 cm, and average height of 27 m.

I. cembrae beetles were trapped using Theysohn® slot barrier traps with Cembräwit® lures. At the Slezské Rudoltice locality, the traps (5 pcs) were placed 10–15 m in front of forest stand edge. Lures were placed in mid-April, just before the beginning of emergence and were renewed eight weeks later (inspection in an interval of 7–10 days).

Healthy larches were cut at the edge of the stand in the second half of March at intervals of 10–15 m for trap trees of a first set (1^{st} set) and at the end of June. The trap trees of a second set (2^{nd} set) (June cutting) were placed inside the stand. Entry holes were counted on four samples of each trap tree. The first study (root) sample was placed in the base part of the trap tree, the second (trunk) sample was in the centre of the trunk between the root and below-crown sample, the third (below-crown) sample was in the place at the beginning of crown, and the fourth (mid-crown) sample in the mid of the crown. Change in abundance according to the number of entry holes was recorded continually at intervals of 7–10 days in the upper part of each sample in a strip (0.1×1 m) of bark divided into ten fields covering an area of 0.01 m^2. Each entry hole was marked by pin. At the Slezské Rudoltice locality, 22 trap trees were inspected (2007–2008), and at the Útěchov locality it was nine trap trees (2008–2009).

At the Slezské Rudoltice locality, always five 0.7 m long logs (Σ 20; August 2008) were obtained from the upper part of four trap trees. Four logs always including one log from each tree representing diagonally the profile of the sampled parts of traps were placed at five sites in the larch stand. From the end of September the sets of sections thus created were gradually placed into emergence traps in laboratory conditions (temperature of 20 °C) in monthly intervals (November–January). At the end of March (2009), emergence traps with area of 0.5 m^2 were placed on ground on each sites. Beetles were sampled in a 14-day interval. In 2008, pairs of emergence traps were placed at the base of larches in a transect line at three sites with an interval of 10 m.

At the Útěchov locality (2008), the number of galleries was determined on all sections of three trap trees and the lengths of maternal galleries were measured, as well as the ten longest larval galleries on each side of the materanal gallery. Furthermore, the numbers of larval galleries on each side of the parent gallery were determined.

The volume of wood infested by *I. cembrae* in the Czech Republic has been documented over the long term (Knížek and Zahradník 1996, Zahradník 1996, 1997, Zahradník and Knížek 1998, 1999, 2000, Knížek 2001, 2002, 2003, Knížek and Zahradník 2004, Knížek 2005, Knížek and Holuša 2006, 2007, Knížek 2008, 2009, 2010).

Numbers of entry holes and number of sampled beetles were compared by nonparametric test (Mann-Whitney U test, Kruskal-Wallis test, median test) that were together with regression analyses performed in program Statistica 9.0.

RESULTS

REZULTATI

Relatively low (but varying year to year) volumes of wood infested by *I. cembrae* in the Czech Republic were recorded during 1996–2009. Increase in wood damaged by bark beetles in 2006 is connected with gradual local increases of *I. cembrae* (Figure 1).

At total, 15,100 and 3,158 beetles were captured in five traps using Cembräwit® pheromone evaporators at Slezské Rudoltice in 2007 and 2008. Flight activity began in the second half of April and ended at the beginning of August (Figure 2). Total numbers of trapped beetles were significantly higher in 2007 than in 2008 (U=1075.5**).

The number of entry holes on trap trees in Slezské Rudoltice locality dropped significantly from spring 2007 to summer 2008, while at the Útěchov locality it significantly increased from spring 2008 to summer 2009 (H (5, N=152) =94.26***; Table 1).

In all cases, only beetles and galleries of *I. cembrae* were determined on trap trees. Entry holes on trap trees samples at the Slezské Rudoltice locality was balanced in individual years. There was no significant difference in the numbers of entry holes of entire trees (H (6, N=28) =13.72, p>0.01; 2008 1st set: H (7, N=32) =10.77, p>0.10; 2008 2nd set: H (4, N= 20) =9.14, p>0.05). Infestation of trap trees at the Útěchov locality was balanced in 2008 and 2009. There was no significant difference in the infestation of entire trees (2008: H (2, N=12) =1.93, p>0.10; 2009 1st set: H (2, N=12) =7.64, p >0.01; 2009 2nd set: H (2, N=12) =3.51, p>0.10).

At Slezské Rudoltice, beetles of the overwintering generation infested trap trees continually from mid May and the offspring generation from mid July, and the number of entry holes increased in the 1st (Table 2) and 2nd trap trees set during 2008 (July 7: 0.0±0.0 entry holes per dm^2; August: 29 0.3±0.6 entry holes per dm^2) (1st set: H (7, N=3520)=754.1***; 2nd set: H (6, N=1400)=57.71***). At the Útěchov locality, beetles of the overwintering generation sought trap trees from mid April with gradual increase in abundance in 2008 (H (10, N=1320)=827.72***) and 2009 (H (10, N=1320)=457.93 ***) (Table 2). Occurrence of entry holes was also recorded on branches 3 cm thick from trap trees of 1st set.

Abundance of *Ips cembrae* entry holes on study sections at the Slezské Rudoltice locality was balanced in almost all cases (2007: H (3, N=28) =9.06, $p>0.01$; 2008 2nd set: H (3, N=20) =5.21, $p>0.10$). The root sections in the 2008 1st set were an exception, having significantly lower abundance (H (3, N=32) =12.56, $p<0.01$; z=3.53**). Abundance on study sections was balanced at the Útěchov locality in all cases (2008: H (3, N=12) =5.69, $p>0.10$; Median test: 2009 1st set: χ^2=3.00, $p>0.10$; 2009 2nd set: χ^2= 6.67, $p>0.10$).

There were significant differences in the length of maternal galleries on samples of the 1st set trap trees (Útěchov, 2008) (H (3, N=78) =38.64***). The difference was caused by significantly longer galleries of the below-crown and mid-crown sections than of the root section (z=3.04*; z=5.57**) and of the mid-crown than of the trunk section (z=4.67**) (Table 3).

There are significant differences in the number of larval galleries on the left side of maternal galleries of study sample (H (3, N=78) =45.23***) because the below-crown and mid-crown sample had significantly lower numbers of larval galleries than did the root sample (z=3.84**; z=6.36**) and the mid-crown sample had more than did the root sample (z=3.84**; z=6.36**) (Table 3). There are significant differences in the numbers of larval galleries on the right side of maternal galleries on study samples (H (3, N=78)=56.39***) because the below-crown and mid-crown samples had significantly lower numbers of larval galleries than did the root sample (z=3.75**; z=7.20**) and the mid-crown sample had more than did the trunk (4.50**) and below-crown samples (z=3.55**) (Table 3).

There are significant differences in the length of larval galleries on the left side of maternal galleries on individual samples (H (3, N=78) =58.40***) because the below-crown and mid-crown samples had significantly longer larval galleries than did the root sample (z=4.21**; z=7.30**) and the mid-crown sample had longer galleries than did the trunk (z=4.98**) and the below-crown (z=3.16**) samples (Table 3). There are significant differences in the lengths of larval galleries on the right side of parent galleries on study samples (H (3, N=78) =58.10***) because the trunk, below-crown and mid-crown samples had significantly longer larval galleries than did the root sample (z=2.66*; z=4.07**; z=7.37**) and the mid-crown sample had longer galleries more than did the below-crown (z=4.82**) and trunk samples (z=3.39**) (Table 3). Lengths of larval galleries on the left and right sides of the maternal gallery did not differ significantly (t=0.44; $p>0.10$).

There were no differences in the total number of beetles that left the logs transferred gradually (from November to December) from the forest to emergence traps in the laboratory (χ^2 = 2.28, $p>0.10$) (Figure 3). *I. cembrae* beetles reacted immediately after being placed into emergence traps in the laboratory and left their place of development within two weeks. Their activity ended after six weeks (Figure 3).

Numbers of *I. cembrae* beetles collected by emergence traps in sites where study logs were placed were quit high (49.3±20.2 adult.m^{-2}) in 2009. The number of beetles leaving study logs did not correlate with the number of beetles captured in emergence traps in sites where study logs were located (r=-0.36, p>0.10). Beetles began to be active at the beginning of May 2009, with culmination in the second half of May. The last beetles were trapped in the first half of June. In contrary, average abundance of beetles overwintering in forest litter of the larch stands was very low (1.0±2.2 adult.m^{-2}) in 2008. In two traps, no beetles were trapped at all.

DISCUSSION

RASPRAVA

Even though sanitary cutting of wood infested with *I. cembrae* is minor in comparison with overall sanitary cutting caused by bark-beetle in the Czech Republic (Zahradník 1997), it is considered a serious pest in some European countries (Grégoire and Evans 2004). Short-term outbreak were set off in central Europe by extreme drought in 2003 (Krehan and Cech 2004, Knížek and Zahradník 2004, Stratmann 2004). In Poland, moreover, its breeding was promoted by wood left after thinning made in young larch forests (Hutka 2006). While wood infested by *I. cembrae* peaked in the Czech Republic in 2006 with subsequent decrease, the amount of wood damaged by bark beetles in Poland increased six fold between 2006 and 2007 (Grodzki and Kosibowicz 2009). Outbreaks are known from the past, e.g. *I. cembrae* occurred on spruce after a *Lymantria monacha* (Linné, 1758) outbreak during the 1920s in the Křivoklát area and the Brdy hills (Pfeffer 1955).

Most often, *I. cembrae* has two generations in a year (Šrot 1976, Michalski and Mazur 1999, Krehan 2004, Knížek 2006, Zúbrik et al. 2008, Grodzki and Kosibowicz 2009). The number of generations depends on the weather (Krehan 2004) and altitude (Grodzki and Kosibowicz 2009). Spring emergence begins in lower and middle altitudes usually at the beginning of May, more rarely at the end of April (Postner 1974, Grodzki and Kosibowicz 2009, present study). Males bore and release a pheromone consisting of ipsdienol, ipsenol and 3-methyl-3-buten-1-ol (Stoakley et al. 1978, Rebenstorff and Francke 1982). In the field, the combination of these three compounds is very attractive (Stoakley et al. 1978). The absolute configuration of ipsenol and ipsdienol from German populations of *I. cembrae* has been determined as 95% (*S*)-(−)-ipsenol and 95% (*S*)-(+)-ipsdienol (Francke and Vité 1983). Interestingly, amitinol, which had been reported as one of the major pheromone components (Kohnle et al. 1988), was not detected in the symplex of *I. subelongatus* (Zhang et al. 2000). Kohnle et al. (1988) detected amitinol in aeration samples of a larch bolt infested by both males and females. Thus, it is not clear whether amitinol is released by feeding beetles (males, females, or both), a host bolt under infestation, or both beetles and bolt (Zhang et al. 2000), but it does significantly enhance field response in Europe to the three-component aggregation pheromone blend (Kohnle et al. 1988). *I. cembrae* also transforms (-)-α-pinene mainly into myrtenol and trans-verbenol, but also into cis-verbenol (Kohnle et al. 1988). Upon exposure to vapour of α-pinene, however, Kohnle et al. (1988) detected myrtenol produced by *I. cembrae* in the headspace. Surprisingly, trace amounts of Vn were detected in later phases of the infestation, which might be a contamination from outside sources, such as autoxidation or microbial activity on the gallery wall (Zhang et al. 2000). *Cis*-verbenol was shown to have a strong inhibitory effect on pheromone response of *I. cembrae* in the field (Kohnle et al. 1988).

Trees infested by bark beetles defend themselves by means of a number of chemical changes in the bast (Terasaki et al. 1987, Jung et al. 1994, Rohde 1995, Rohde et al. 1996). Fungi that are transmitted by the beetles and that cause blue stains in the wood (Redfern 1989, Kirisits et al. 2000, Jankowiak et al. 2007) help the beetles overcome the defensive reactions of trees, which, therefore, makes them technical pests as well.

Beetles of the second generation emerged at the turn of June/July, when pupae and callow beetles were found on trap trees. Flight activity was also recorded much later, however, with the beginning of flight of the offspring generation (Postner 1974) and also in lower altitudes (Grodzki and Kosibowicz 2009). There may also be a re-emerging beetles of the overwintering generation, flying in June. The offspring generation adults have a maturation feed in late summer, either in branches of younger trees (observed) or near to the larval galleries, if there is still fresh bark present. Adults aggregate in response to terpenoid pheromones (Kohnle et al. 1988). Adults overwinter partly in tunnels resulting from maturation feeding under the thicker bark of trunks lying on the ground or, more commonly, in the forest litter (Schneider 1977). In case of incomplete development, they may overwinter in larva or pupae (Postner 1974), their success of overwintering depending on the course of the weather in winter.

The proportion of the population overwintering in the imagine stage and the place of overwintering depend upon the weather in autumn and the possibility for completing the entire development. Part of the early breeding beetles will leave the place of development and overwinter in forest litter or stay in the place of feeding. For that reason, some findings document overwintering in the place of feeding (Krehan and Cech 2004) and others in forest litter (Schneider 1977, Grodzki and Kosibowicz 2009). We have proven the presence of overwintering beetles in logs in winter and their immediate activity after being transferred to laboratory conditions. Protracted leaving of study logs evidences that part of the population had overwintered in larva and pupae.

The tendency to overwinter in forest litter is clear, and its explanation lies in lower mortality there than in overwintering on a trunk [see *Ips typographus* (Linné, 1758)] (Wermelinger 2004). Beetles overwinter near the trees where they completed their development. Their abundance was much higher in emergence traps placed by study logs (2009) than in those placed randomly in the forest stand (2008), although the abundance of beetles on trap trees in previous year (2007) was much higher than in 2008. Also in *I. typographus*, 80% of adults overwinter in forest litter directly under the infested trunk or in its vicinity (Zumr 1985). Overwintering beetles begin to be active in May, according to the observed flight activity.

Occupation of the trap trunk profile was even in most cases, but sometimes a higher abundance was observed in the top part. Samples that are higher in the trunk profile of the tree trap are characterized by longer maternal galleries with lower numbers of laid eggs and larval galleries. In *I. subelongatus*, the number of egg niches and adult offspring produced per gallery were shown to decline exponentially with infestation density (Zhang et al. 1992). The exponential relationships were attributed to intraspecific competition between female adult parents for breeding space and between larvae for limited food resources. Inasmuch as the density of feedings on trap trees was identical, females creating parent galleries reacted to thinner phloem by decreased oviposition in galleries, increased distance between laid eggs in order to allow larvae to obtain adequate food by creating longer larval galleries.

Examination confirmed the fact that *Ips cembrae* virtually has no spatial cambioxylophagous competitor in central Europe. *Tetropium gabrieli* Weise, 1905 or possibly *Pityogenes chalcographus* (Linné, 1761) can develop in parallel on the same tree, and *Pityophthorus pityographus* (Ratzeburg, 1837) and *Cryphalus intermedius* Ferrari, 1867 on thinner branches (Knížek 2006).

Control of *I. cembrae* is identical with that for other species of bark beetles that live in conifers. In European countries, it is used in varying intensity depending on the severity of damage:

(i) silviculture management: clear cutting, selective thinning, silvicultural selection;

(ii) chemical treatment of felled trees;

(iii) trapping out measures: trap trees, trap logs, pheromone traps, baited slash;

(iv) monitoring by pheromone attractants, trap trees/logs, visual survey, questionnaire (Grégoire and Evans 2004).

In Europe, four types of pheromone evaporators are used at present: Cembräwit® (www.witasek.com), Cemprax (Shell Agrar Ltd.) (www.witasek.com), Cemsan (www.fluegel-gmbh.de) and Cembrodor (Glowacka 2008). The pheromone lure Cembräwit® serves for exclusive trapping of *I. cembrae*; when inspecting pheromone traps, no presence of other representatives of the under-bark fauna was recorded. In Slovenia, the pheromone evaporator Cemprax® was used for trapping *I. cembrae* while proving effective also for other species of cambial fauna. *Hylastes cunicularius* Erichson, 1836, *P. chalcographus, Dryocoetes autographus* Ratzeburg, 1837, *I. typographus* and *Ips acuminatus* (Gyllenhal, 1827) occurred among the trapped beetles in larger numbers. *I. cembrae* represented 98% of the total catch (Pavlin 2001).

Tests under laboratory conditions showed best breeding success of *I. cembrae* in larch timber felled during February–March. In field experiments, timber from the April felling was infested first and invaded severely (Elsner 1997). Traps from trees of $d_{1.3}$ 30–45 cm prepared in this study in the second half of March on sunlit places were occupied very intensively. Trap trees can remain active until the first pupae occur, because even when there was a great abundance of entry holes invasion on trap trees was not halted. On the other hand there is a possibility of re-emerging of the beetles that infested trap trees firstly.

At the Slezské Rudoltice locality, trapping of adults in pheromone and trap trees decreased substantially (2008) as a result of protective measures implemented (pheromone traps, trap trees, timely elimination of infested trees) in 2007.

In comparison with other European bark beetles of the *Ips* genus, there are persisting problems in protecting against *I. cembrae*: (i) it develops on branches, (ii) a considerable part of the population can overwinter in forest litter (also in other *Ips* species), and (iii) trees processed by harvesters are not protected against infestation.

(i) *I. cembrae* also develops on branches that are up to 3 cm thick (see also Knížek 2006). This knowledge must be taken into account in applying protective methods, and it is necessary to dispose of logging residues and brushwood (burning and chipping) so that no feedings can be created and no breeding of this species occurs in this material. Unprocessed logging waste from thinning interventions in young stands is invaded as well.

(ii) Since *I. cembrae* can overwinter in forest litter, removal of trees infested by bark beetles may not be sufficiently effective in decreasing the population density. Extension of the vegetation period by favourable temperatures increases the proportion of adults overwintering in forest litter, and that intensifies the threat of spring larch infestation.

(iii) Larch harvested by modern harvesters can be successfully colonized by *I. cembrae* – or at least those parts having thick bark – and accordingly, appropriate control is necessary. When thinning wood is left debarked in the stand, infestations may become severe, so spatial or temporal gaps should be left between harvesting and thinning (Watzek and Niemeyer 1996).

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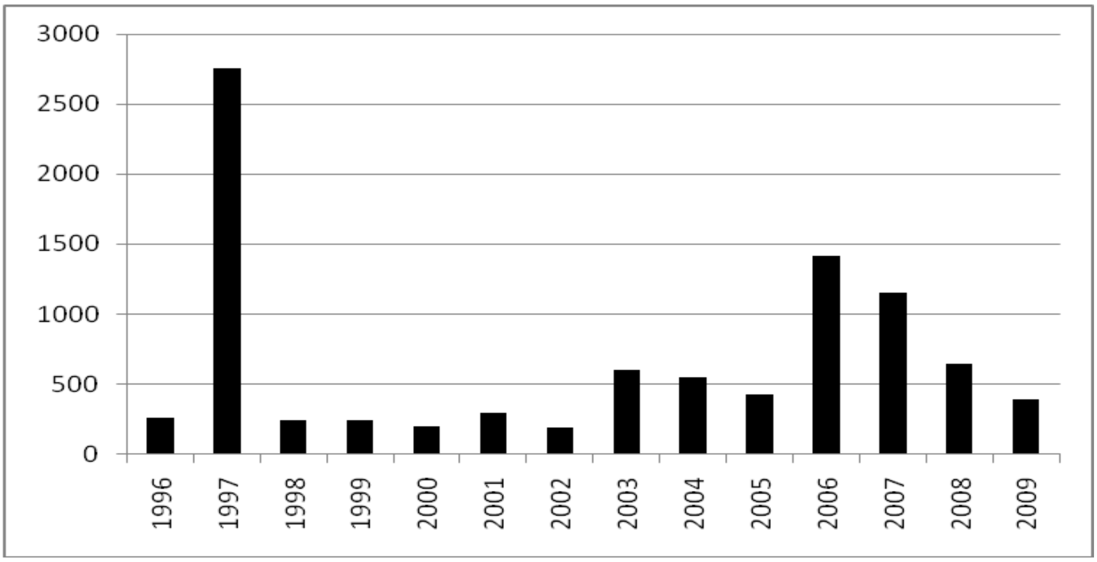

Figure 1 Volume of wood damaged by *Ips cembrae* in the Czech Republic (1996–2009)

Slika 1 Voumen drveta uništen od Ips cembare u Češkoj (1996-2009)

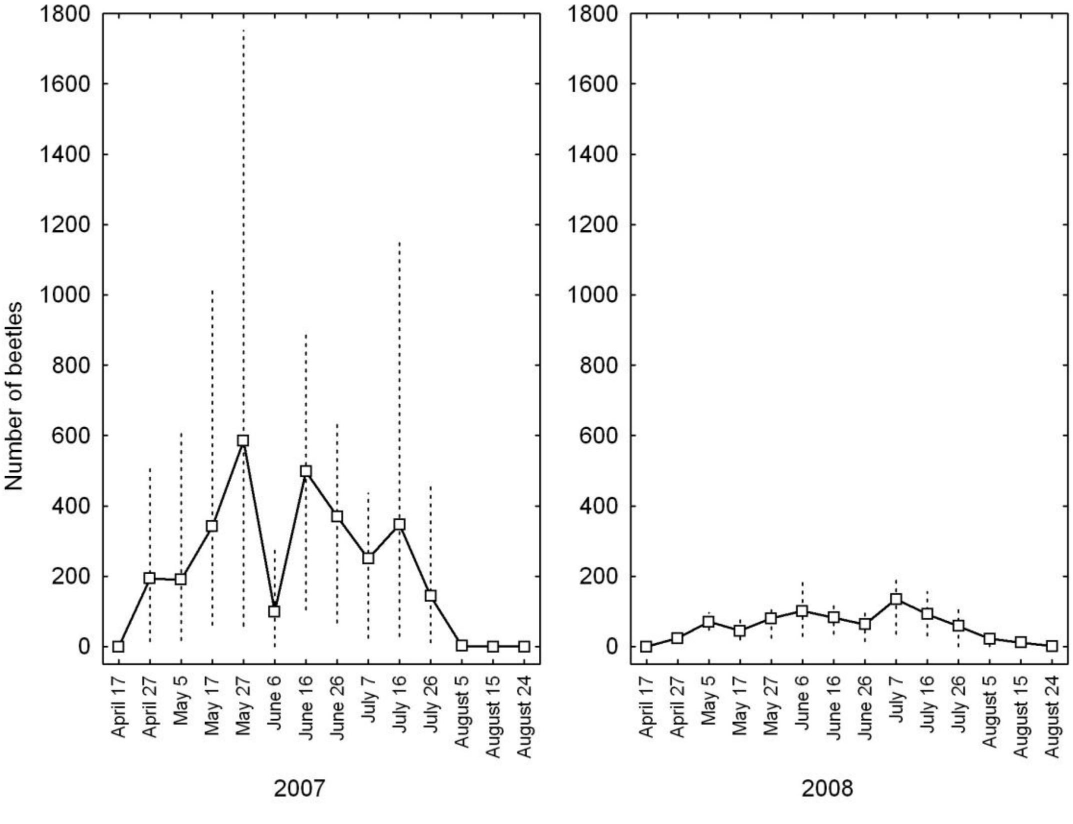

Figure 2 Seasonal flight activity of *Ips cembrae* based on the trapping of beetles (Slezské Rudoltice, 2007–2008) (Square...mean numbers of sampled beetles; dotted line...SD)

Slika 2 Sezonsko rojenje Ips cembrae na temelju ulova kornjaša (Slezské Rudoltice, 2007–2008) (kvadrat...srednji broj sakupljenih kornjaša; točka crta...SD)

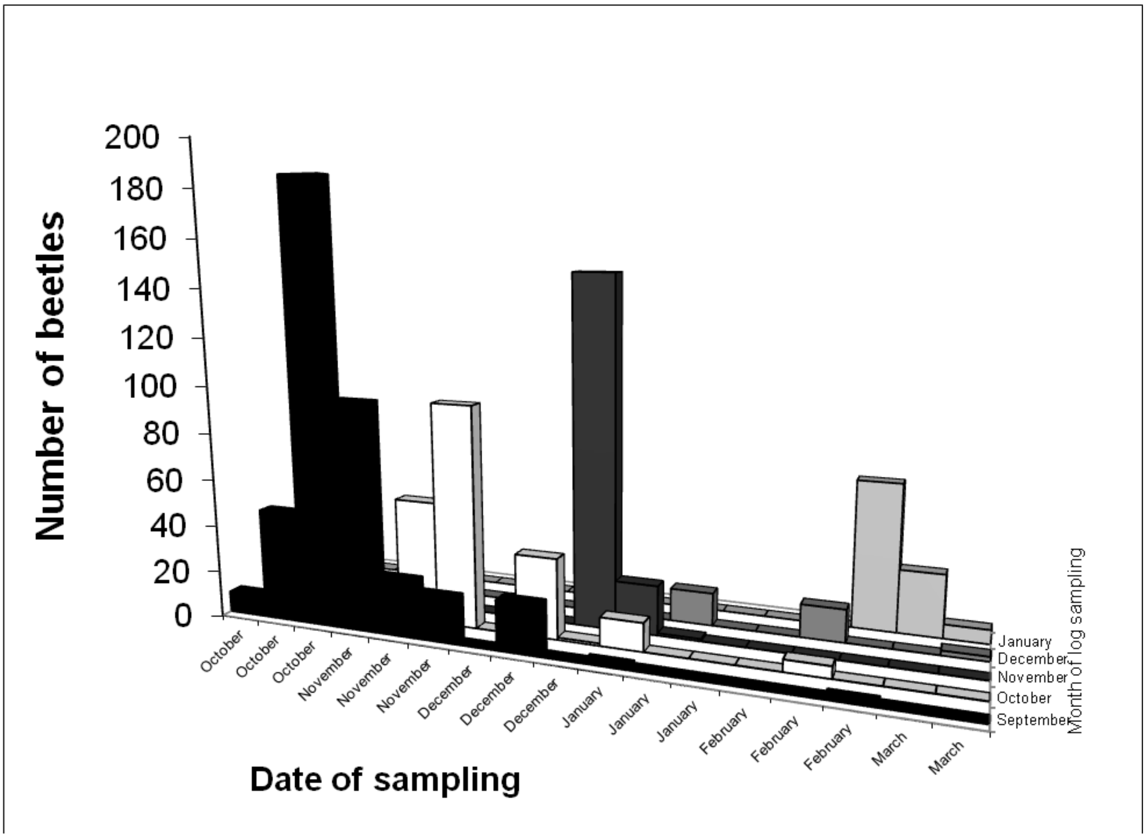

Figure 3 Time distribution of *Ips cembrae* beetles leaving in laboratory (month of log sampling: black–September, white–October, dark grey–November, lighter grey–December, light grey–January)

Slika 3 Vremenska distribucija iztlazečih Ips cembrae *u laboratoriju (sakupljanje trupčića po mjesecima: crno-rujan; bijelo-listopad; tamnosiva-studeni; svijetlijesiva-prosinac; svijetlosiva-siječanj)*

Table 1 Total abundance of entry holes *Ips cembrae* (per 1dm^2) on trap trees and multiple comparisons by Kruskall-Wallis test

Tablica 1 Ukupna abundanca ulaznih rupa Ips cembrae (po 1dm^2) na lovnimmstablima i višestruka nusporedba Kruskall Wallis testom

Study	Locality	Year	Period	Number of trap trees	Number of entry holes (per dm2±SD)	1	2	3	4	5	6
1	Slezské Rudoltice	2007	April-June	7	3.2±1.4		5.21n.s.	7.53n.s.	1.50n.s.	0.77n.s.	2.40n.s.
2	Slezské Rudoltice	2008	April-June	8	1.4±0.6	5.21***		3.38n.s.	1.75n.s.	3.49n.s.	5.57n.s.
3	Slezské Rudoltice	2008	July-August	5	0.3±0.4	7.53***	3.38*		4.06n.s.	5.39n.s.	7.46n.s.
4	Útěchov	2008	April-June	3	2.3±0.6	1.50n.s.	1.75n.s.	4.06***		1.69n.s.	3.04n.s.
5	Útěchov	2009	April-June	3	3.4±1.2	0.77n.s.	3.49**	5.39***	1.69n.s.		1.03n.s.
6	Útěchov	2009	July-August	3	5.1±1.8	2.40n.s.	5.57***	7.46***	3.04*	1.03n.s.	

Table 2 Abundance of entry holes *Ips cembrae* (per 1dm^2) during season

Tablica 2 Abundanca ulaznih rupa Ips cembrae (po 1dm^2) u sezoni

Locality	Year	April 10	April 18	April 26	May 7	May 17	May 24	May 31	June 6	June 13	June 21
Slezské Rudoltice	2008	0±0	0±0	0±0	0±0	0.3±0.6	0.4±0.7	0.7±0.9	0.8±1.0	1.0±1.0	1.0±1.1
Útěchov	2008	0±0	0±0	0.8±0.7	1.2±0.8	1.5±0.5	1.9±1.0	2.1±1.1	2.2±1.1	2.3±1.0	2.3±1.1
Útěchov	2009	0±0	0±0	0.3±0.5	0.5±0.6	0.7±0.3	1.4±1.3	1.7±1.5	1.9±1.6	2.1±1.7	2.3±2.0

Table 3 Length of maternal galleries, numbers and lengths of larval galleries (±SD) of *Ips cembrae* depending on place of sample on trap tree

Tablica 3 Dužina materinskih hodnika, broj i dužina larvalnih hodnika (±SD) Ips cembrae ovisno o mjestu sakupljanja na lovnom stablu

	Side of parental gallery	Root	Trunk	Below-Crown	Mid-Crown
Lenght of parent galleries (cm)		10.7±1.6	11.5±1.9	13.0±1.5	14.5±1.4
Number of larval galleries (per dm^2)	Left	19.4±3.7	15.7±1.6	12.5±1.5	12.5±1.5
	Right	19.7±3.1	15.4±1.5	11.8±1.1	11.8±1.1
Length of larval galleries (cm)	Left	4.0±0.2	4.4±0.4	4.7±0.3	5.3±0.2
	Right	3.9±0.2	4.4±0.3	4.6±0.3	5.3±0.3

REFERENCES

LITERATURA

Bevan, D., 1987: Forest insects. A guide to insects feeding on trees in britain. Forestry Commission, Handbook 1. HMSO, 153, London.

Elsner, G., 1997: Relationships between cutting time in winter and breeding success of *Ips cembrae* in larch timber. Mitt. Dtsch. Ges. Allg. Angew. Ent., 11: 653–657.

Francke, W., J. P. Vité, 1983: Oxygenated terpenes in pheromone systems of bark beetles. Z. Angew. Entomol., 96: 146–156.

Glowacka, B., 2008: Srodki ochrony roslin zalecane do stosowania w lesnictwie w roku 2009. Inst. Badaw. Lesn., analizy i raporty, 11: 1–68.

Grégoire, J.-C., H. F. Evans, 2004: Damage and control of BAWBILT organisms – an overview. In: Lieutier, F., K. R. Day, A. Battisti, J.-C. Grégoire, H. F. Evans, (EDS), Bark and wood boring insects in living trees in Europe, a synthesis. Kluwer Academic, 19–37, Dordrecht.

Grodzki, W., 2008: *Ips cembrae* Heer. (Col.: Curculionidae, Scolytinae) in young larch stands – a new problem in Poland. Forstsch. Aktuell, 44: 8–9

Grodzki, W., M. Kosibowicz, 2009: Materiały do poznania biologii kornika modrzewiowca *Ips cembrae* (Heer) (Col., Curculionidae, Scolytinae) w warunkach południowej Polski. Sylwan, 153: 587–593.

Hutka, D., 2006: Nowe oblicze kornika modrzewiowca. Trybuna Leśnika, 4: 10–11.

Jankowiak, R., R. Rossa, K. Miśta, 2007: Survey of fungal species vectored by *Ips cembrae* to European larch trees in Raciborskie forests (Poland). Czech Mycol., 59: 227–239.

Jung, P., M. Rohde, J. Lunderstadt, 1994: Induzierte Resistenz im Leitgewebe der Europaischen Larche *Larix decidua* Mill nach Befall durch den Grossen Larchenborkenkäfer *Ips cembrae* Heer (Col.: Scol.) J. Appl. Entomol., 117: 427–433.

Kirisits, T., R. Grubelnik, E. Führer, 2000: Die ökologische Bedeutung von Bläuepilzen für rindenbrütende Borkenkäfer. FBVA Berichte, 111: 117–137.

Knížek, M., 2001: Podkorní hmyz. In: Kapitola, P., M. Knížek, (EDS), Výskyt lesních škodlivých činitelů v roce 2000 a jejich očekávaný stav v roce 2001. Zprav. Ochr. Lesa (Suppl.), VÚLHM, Jíloviště – Strnady, 76: 17–27.

Knížek, M., 2002: Podkorní hmyz. In: Kapitola, P., M. Knížek, (EDS), Výskyt lesních škodlivých činitelů v roce 2001 a jejich očekávaný stav v roce 2002. Zprav. Ochr. Lesa (Suppl.), VÚLHM, Jíloviště – Strnady, 68: 15–24.

Knížek, M., 2003: Podkorní hmyz. In: Kapitola, P., M. Knížek, (EDS), Výskyt lesních škodlivých činitelů v roce 2002 a jejich očekávaný stav v roce 2003. Zprav. Ochr. Lesa (Suppl.), VÚLHM, Jíloviště – Strnady, 64: 15–24.

Knížek, M., 2005: Podkorní hmyz. In: Kapitola, P., P. Baňař, (EDS), Výskyt lesních škodlivých činitelů v roce 2004 a jejich očekávaný stav v roce 2005. Zprav. Ochr. Lesa (Suppl.), VÚLHM, Jíloviště – Strnady, 72: 18–26.

Knížek, M., 2006: Lýkožout modřinový *Ips cembrae* (Heer). Lesn. Práce, 12: Appendix, I−IV.

Knížek, M., 2008: Podkorní hmyz. In: Knížek, M., V. Pešková, (EDS), Výskyt lesních škodlivých činitelů v roce 2007 a jejich očekávaný stav v roce 2008. Zprav. Ochr. Lesa (Suppl.), VÚLHM, Jíloviště – Strnady, 74: 21–33.

Knížek, M., 2009: Podkorní hmyz. In: Knížek, M., (ED), Výskyt lesních škodlivých činitelů v roce 2008 a jejich očekávaný stav v roce 2009. Zprav. Ochr. Lesa (Suppl.), VÚLHM, Jíloviště – Strnady, 72: 20–31.

Knížek, M., 2010: Monitoring lýkožrouta severského v Česku v roce 2009. Lesn. Práce, 89: 46–47.

Knížek M., J. Holuša, 2006: Podkorní hmyz. In: Kapitola, P., (ED), Výskyt lesních škodlivých činitelů v roce 2005 a jejich očekávaný stav v roce 2006. Zprav. Ochr. Lesa (Suppl.), VÚLHM, Jíloviště – Strnady, 76: 20–31.

Knížek M., J. Holuša, 2007: Podkorní hmyz. In: Knížek, M., (ED), Výskyt lesních škodlivých činitelů v roce 2006 a jejich očekávaný stav v roce 2007. Zprav. Ochr. Lesa (Suppl.), VÚLHM, Jíloviště – Strnady, 74: 21–32.

Knížek, M., P. Zahradník, 1996: Mass outbreak of *Ips duplicatus* Sahlberg (Coleoptera, Scolytidae). XX. International Congress of Entomology – Proceedings, Italy, 25-31 August, 527, Firenze.

Knížek M., P. Zahradník, 2004: Podkorní hmyz. In: Kapitola, P., M. Knížek, P. Baňař, (EDS), Výskyt lesních škodlivých činitelů v roce 2003 a jejich očekávaný stav v roce 2004. Zprav. Ochr. Lesa (Suppl.), VÚLHM, Jíloviště – Strnady, 80: 30–39.

Kohnle, U., J. P. Vité, C. Erbacher, J. Bartels, W. Francke, 1988: Aggregation response of European engraver beetles of the genus *Ips* mediated by terpenoid pheromones. Entomol. Exp. Appl., 49: 43–53.

Krehan, H., 2004: Data sheet: *Ips cembrae* (Großer Lärchenborkenkäfer). Forstsch. Aktuell, 32: 9.

Krehan, H., T. L. Cech, 2004: Larch damage in Upper Styria. An example of the complex effects of damage agents. Forstsch. Aktuell, 32: 4–8.

Krehan, H., G. Steyer, 2005: Borkenkäfer-Monitoring und Borkenkäfer-kalamität 2004. Forstsch. Aktuell, 33: 12–14.

Luitjes, J., 1974: *Ips cembrae*, a new noxious forest insect in the Netherlands. Ned. Bosb. Tijdschr., 46: 244–246.

Michalski, J., A. Mazur, 1999: Korniki. Praktyczny przewodnik dla leśników. Wydawnictwo Świat, 188, Warszawa.

OEPP/EPPO, 2005: *Ips cembrae* and *Ips subelongatus*. Bull. OEPP/EPPO, 35: 445–449.

Pavlin, R., 2001: The catch of the larch bark beetle *Ips cembrae* (Heer) with pheromone traps in Slovenia. J. For. Sci., 47 (Special Issue 2): 143–146.

Pfeffer, A., 1955: Kůrovcovití – Scolytoidea (řád brouci – Coleoptera). Fauna ČSR sv. 6, NČSAV, 324, Praha.

Pfeffer, A., 1989: Kůrovcovití Scolytidae a jádrohlodovití Platypodidae. Academia, 138, Praha.

Pfeffer, A., 1995: Zentral- und westpaläarktische Borken-und Kernkäfer (Coleoptera: Scolytidae, Platypodidae). Pro Entomologia, 310, Basel.

Postner, M., 1974: *Ips cembrae*. In: Schwenke, W., (ED), Die Forstschädlinge Europas. II. Band. Käfer. Paul Parey, 458–459, Hamburg.

Rebenstorff, H., W. Francke, 1982: The large larch bark beetle: monitoring with attractants? Allg. Forst Z., 37: 450.

Redfern, D. B., 1989: The roles of the bark beetle *Ips cembrae*, the woodwasp *Urocerus gigas* and associated fungi in dieback and death of larches. In: Wilding, N., N. M. Collins, P. M. Hammond, J. F. Webber, (EDS), Insect fungus interactions, 14th symposium of the Royal Entomological Society of London in collaboration with the British Mycological Society. Academic Press, 195–204, London.

Rohde, M., 1995: Physiological investigations into susceptibility, defence reactions and resistance, in relations between larch and the larch bark beetle (*Ips cembrae*). Mitt. Dtsch. Ges. Allg. Angew. Ent., 10: 51–54.

Rohde, M., R. Waldmann, J. Lunderstandt, 1996: Induced defence reaction in the phloem of spruce (*Picea abies*) and larch (*Larix decidua*) after attack by *Ips typographus* and *Ips cembrae*. Forest Ecol. Manag., 86: 51–59.

Schneider, H. J., 1977: Experience in the control of the large larch bark beetle in stands of low vitality. Allg. Forst Z., 32: 1115–1116.

Šrot, M., 1976: Některé nové poznatky o zakládání sesterského pokolení lýkožrouta modřínového (*Ips cembrae* Heer.) v ČSR. Lesnictví, 22: 979–996.

Stauffer, C., T. Kirisits, C. Nussbaumer, R. Pavlin, M. J. Wingfield, 2001: Phylogenetic relationships between the European and Asian eight spined larch bark beetle populations (Coleoptera, Scolytidae) inferred from DNA sequences and fungal associates. Eur. J. Entomol., 98: 99–105.

Stoakley, J. T., A. Bakke, J. A. A. Renwick, J. P. Vité, 1978: The aggregation pheromone system of the larch bark beetle, *Ips cembrae* Heer. Z. Angew. Entomol., 86: 174–177.

Stratmann, J., 2004: Borkenkäferkalamitt 2003 was haben wir gelernt, sind wir für 2004 gerüstet? Forst Holz, 59: 166–169.

Suzuki, S., H. Imada, 1993: Effect of temperatures on the developmental period of *Ips cembrae* (Heer) (Coleoptera: Scolytidae). J. Jap. Forestry Soc., 75: 538–540.

Terasaki, Y., N. Yosida, K. Fukuyama, K. Furuta, 1987: Response of *Larix leptolepis* to inoculated *Ips cembrae*. Bull. Tokyo. Univ. For., 77: 19–30.

Watzek, G., H. Niemeyer, 1996: Verminderung der Borkenkäfergefähr durch Harvestertechnik und Arbeitsorganisation. Forst Holz, 51: 247–250.

Wermelinger, B., 2004: Ecology and management of the spruce bark beetle *Ips typographus* – a review of recent research. Forest Ecol. Manag., 202: 67–82.

Westhuizen van der, K., M. Wingfield, Y. Yamaoka, G. H. J. Kemp, P. W. Crous, 1995: A new species of *Ophiostoma* with a *Leptographium* anamorph from larch in Japan. Mycol. Res., 99: 1334–1338.

Yamaguchi, T., K. Sasaki, S. Matsuzaki 1989: Reaction of Japanese larch inoculated with *Ceratocystis piceae*. Annual Report of the Hokkaido Research Center, Forestry and Forest Products Research Institute, 75–79, Hokkaido.

Yamaoka, Y., M. J. Wingfield, M. Ohsawa, Y. Kuroda, 1998: Ophiostomatoid fungi associated with *Ips cembrae* in Japan and their pathogenicity to Japanese larch. Mycoscience, 39: 367–378.

Zahradník, P., (ED), 1996: Výskyt lesních škodlivých činitelů v roce 1995 a jejich očekávaný stav v roce 1996. Zprav. Ochr. Lesa (Suppl.), VÚLHM, Jíloviště – Strnady, 1996: 1–64.

Zahradník, P., (ED), 1997: Výskyt lesních škodlivých činitelů v roce 1996 a jejich očekávaný stav v roce 1997. Zprav. Ochr. Lesa (Suppl.), VÚLHM, Jíloviště – Strnady, 1997: 1–88.

Zahradník, P., M. Knížek, 1998: Podkorní hmyz. In: Zahradník, P., (ED), Výskyt lesních škodlivých činitelů v roce 1997 a jejich očekávaný stav v roce 1998. Zprav. Ochr. Lesa (Suppl.), VÚLHM, Jíloviště – Strnady, 1998: 1–88.

Zahradník, P., M. Knížek, 1999: Podkorní hmyz. In: Knížek, M., P. Kapitola, (EDS), Výskyt lesních škodlivých činitelů v roce 1998 a jejich očekávaný stav v roce 1999. Zprav. Ochr. Lesa (Suppl.), VÚLHM, Jíloviště – Strnady, 1999: 13–21.

Zahradník, P., M. Knížek, 2000: Podkorní hmyz. In: Knížek, M., P. Kapitola, (EDS), Výskyt lesních škodlivých činitelů v roce 1999 a jejich očekávaný stav v roce 2000. Zprav. Ochr. Lesa (Suppl.), VÚLHM, Jíloviště – Strnady, 2000: 16–23.

Zhang, Q. H., G. Birgersson, F. Schlyter, F. Chen-Guo, 2000: Pheromone components in the larch bark beetle, *Ips cembrae*, from China: quantitative variation among attack phases and individuals. J. Chem. Ecol., 26: 841–858.

Zhang, Q. H., J. A. Byers, F. Schlyter, 1992: Optimal attack density in the larch bark beetle, *Ips cembrae* (Coleoptera: Scolytidae). J. Appl. Ecol., 29: 672–678.

Zhang, Q. H., F. Schlyter, F. Chen-Guo, Y. J. Wang, 2007: Electrophysiological and behavioral responses of *Ips subelongatus* to semiochemicals from its hosts, non-hosts, and conspecifics in China. J. Chem. Ecol., 33: 391–404.

Zúbrik, M., A. Kunca, J. Novotný, 2008: Hmyz a huby. Národné lesnícke centrum, 178, Zvolen.

Zumr, V., 1985: Biologie a ekologie lýkožrouta smrkového (*Ips typographus*) a ochrana proti němu. Academia, 105, Praha.

SAŽETAK

U čitavom arealu, od najniže nadmorske visine do subalpinskog pojasa, glavni domaćin potkornjaku *Ips cembrae* je europski ariš, *Larix decidua* Mill. Samo ponekad ova vrsta naseljava i smreku, *Picea abies* (L.) Karsten. Osnovna bionomija potkornjaka *I. cembrae* je više-manje poznata i razlikuje se od ostalih vrsta roda *Ips*. Unatrag nekoliko godina publicirano je tek nekoliko istraživanja vezano za bionomiju i zaštitu šuma od ovog štetnika. Cilj ovog rada je stjecanje novih saznanja o:

(i) Rojenju, kroz pokus korištenja sintetskih mamaca; kornjaši *I. cembrae* su se lovili pomoću Theysohn® naletnih klopki te korištenjem feromonskih pripravaka Cembräwit®;

(ii) prostornoj i vremenskoj distribuciji kornjaša na lovnim stablima; u drugom dijelu ožujka zdrava stabla ariša su obarana radi postavljanja prvog seta lovnih stabala u razmaku od 10-15 m na rubovima sastojina. Krajem srpnja drugi set postavio se unutar sastojine. Ulazne rupe su evidentirane na četiri sekcije svakog lovnog stable;

(iii) prezimljavanju kornjaša; pet trupčića dužine 0,7 m dobivene iz gornjeg dijela četiriju lovnih stabala. Četiri trupčića dobiveni iz svakog lovnog stabla postavljena su na pet mjesta u ariševoj sastojini. Od kraja rujna u mjesečnim intervalima (listopad-siječanj), setovi sekcija postepeno su se premještali u kaveze u laboratoriju.

Sanitarna sječa stabala zaraženi potkornjacima *I. cembrae* mala je u odnosu sa ukupnu sanitarnu sječu koje se provode radi napada potkornjaka u Češkoj U nekim europskimm zemljama takvi napadi predstavljaju ozbiljan problem. Primjerice ekstremna suša u 2003. godini uzrokom je gradacija u Srednjoj Europi. Dok je drvna masa zaražena potkornjacima *I. cembrae* u Češkoj kulminirala u 2006. godini sa trendom opadanja (Slika 1, Tablica 1), količina potkornjacima zaražene drvne mase u Poljskoj povećana je šest puta između 2006. i 2007. godine.

Potkornjak *I. cembrae* najčešće ima 2 generacije godišnje (Slika 2). Proljetno izlaženje počinje na nižim i srednjim nadmorskim visinama početkom svibnja, rjeđe krajem travnja. Potkornjaci druge generacije izlaze na prijelazu lipnja i srpnja. Nova generacija imaga obavlja dopunsko žderanje u kasno ljeto u granama mladih stabala ili u blizini larvalnog hodnika gdje ima svježe kore. Nakon dopunskog žderanja imaga prezimljavaju dijelom u hodnicima ispod tanke kore oblovine ili češće u šumskoj stelji.

U slučaju kad se ne stignu potpuno razviti, prezimljavaju kao ličinka ili kao kukuljica. Tada njihovo preživljavanje ovisi o vremenskim prilikama u zimi. Razmjer populacije koja prezimljava u stadiju imaga i njihova niša prezimljavanja ovisi o vremenskim prilikama u jeseni i uvjetima završavanja razvoja. Dio potkornjaka koji rano završi razvojne stadije napušta mjesto razvoja i prezimljava u šumskoj stelji ili ostaje na mjestu gdje se hrani. Ispitali smo prisutnost prezimljujućih potkornjaka u trupčićima u zimi i njihovu aktivnost nakon što budu premješteni u laboratorijske uvjete (slika 3).

Produženo vrijeme napuštanja pokusnih trupčića potvrdilo je kako dio populacije prezimljuje kao larva i kukuljica. Potkornjaci koji prezimljavaju u stelji to čine blizu stabala na kojem završavaju svoj razvoj.

Potkornjaci prve generacije naseljavaju lovna stabla kontinuirano od polovice svibnja, a druge generacije od polovice srpnja (Tablica 2). Naseljavanje donjih dijelova stabla bilo je jednoliko u gotovo svim slučajevima, a samo je ponekad veća abundanca nađena u gornjem dijelu.

Sekcije iz gornjeg dijela debla lovnog stabla karakteriziraju dulji materinski hodnici sa manjem brojem položenih jaja i larvalnih hodnika (Tablica 3). Eksponencijalni odnos pridaje se interspecifičnoj kompeticiji prostora za polaganje jaja između ženki i ograničenog resursa hrane između ličinki. Kako je gustoća hodnika na lovnim stablima bila jednolika, ženke su u tanjem floemu reagirale manjim brojem položenih jaja i povećavanjem udaljenosti između materinskih hodnika. Na taj način larvama su omogućile izradu dužih larvalnih hodnika i pristup adekvatnoj hrani.

Kontrola potkornjaka *I. cembrae* je identična sa drugim vrstama potkornjaka koji dolaze na četinjačama i obavlja se:

(i) uzgojnim zahvatima: čistom sječom, selektivnom proredom, selekcijom;

(ii) kemijskim tretiranjem oborenih stabala;

(iii) mjerama izlova: lovnim stablima, lovnom oblovinom, klopkama, lovnom sječkom;

(iv) monitoringom uz pomoć feromonskih atraktanata, lovnih stabla/oblovine, vizualnim nadzorom, upitnicima.

U Europi se koriste četiri tipova feromonmskih pripravaka: Cembräwit® (www.witasek.com), Cemprax (Shell Agrar Ltd.) (www.witasek.com), Cemsan (www.fluegel-gmbh.de) i Cembrodor. Feromonski pripravak Cembräwit® se koristi za ciljani ulov *I. cembrae*; tijekom pokusa i pregledavanjem ulova feromonskih klopki nisu zabilježeni ulovi neciljane floemofaune.

U terenskim pokusima, drvo posječeno u travnju prvo jer bilo napadnuto i intenzivno naseljavano. Za vrijeme pokusa lovna stabla promjera $d_{1.3}$ 30–45 cm pripremljena u drugoj polovici ožujka na osunčanoj poziciji, bilo je vrlo intenzivno napadnuto, a lovna stabla su ostala aktivna sve do prve kukuljice. Bez obzira na veliku abundancu ulaznih rupa, naseljavanje lovnih stabala nije se zaustavilo. Moguće je i da su se prethodno ubušeni potkornjaci ponovno ubušivali.

U usporedbi sa drugim europskim vrstama potkornjaka roda *Ips*, evidentan je problem zaštite protiv *I. cembrae* iz razloga što se: (i) razvija u granama; (ii) određen dio populacije može prezimiti u šumskoj stelji (kao i druge *Ips* vrste), i (iii) stabla obrađena harvesterom nisu zaštićena protiv napada.

(i) *I. cembrae* se razvija u granama koji su od 3 cm promjera. To se mora uzeti u obzir prilikom poduzimanja zaštitnih mjera. Uz to treba uništiti drvne ostatke i izbojke (paljenjem i malčiranjem) kako ne bi došlo do žderanja i rasploda štetnika u tom materijalu. Prorjedama u mladim sastojinama nezbrinuti drveni ostaci također budu napadnuti.

(ii) Kako *I. cembrae* može prezimiti u šumskoj stelji, uklanjanje stabala napadnutih potkornjacima, vjerojatno nije dovoljna efektna mjera u smanjivanju gustoće populacije. Produljena vegetacijski period te povoljne temperature povećavaju broj imaga koji prezimljuju u šumskoj stelji što nadalje intenzivira opasnost od napada u proljeće.

(iii) Ariš nakon obrade modernim harvesterima u proljeće može biti uspješno koloniziran jedinkama *Ips cembrae*, prije svega dijelova sa tankom korom, što znači da je zaštita i dalje potrebna. Kada prorjedom materijal dodatno ostaje u sastojini, napad postaje ozbiljniji, zbog čega treba ostaviti prostorni ili vremenski razmak između sječe i prorjede.

***Ključne riječi*: *Ips cembrae*, lovno stablo sa feromonskim pripravkom, lovno stablo, rojenje, disperzija, prezimljavanje**

4.5 Pathogens of the bark beetle *Ips cembrae*: microsporidia and gregarines known from other *Ips* species

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ORIGINAL CONTRIBUTION

Pathogens of the bark beetle *Ips cembrae*: microsporidia and gregarines also known from other *Ips* species

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Abstract

The objective of the current study was to identify pathogens of the large larch bark beetle, *Ips cembrae*, which is a secondary pest that has produced several local outbreaks across Europe in recent years. Beetles were collected from pheromone traps, trap trees and emergence traps (*Larix decidua*) during 2007 to 2011 at 10 study sites in central Europe. A total of 3379 mature and callow beetles were examined with a light microscope, and only two microsporidian pathogens [*Chytridiopsis typographi* and a diplokaryotic microsporidium (probably *Nosema* sp.)] and two gregarines (*Gregarina typographi* and *Mattesia schwenkei*) were found. Within the *I. cembrae* populations, the infection rate for *C. typographi* ranged from 2 to 58%. *Nosema* sp. occurred in only two beetles in 2007 (at two study sites). *G. typographi* was recorded only in Austria and Croatia and only in 1–2% of the beetles in those countries. *Mattesia schwenkei* was observed solely in Croatia in 0.6% of the beetles in that country. Only one fungal pathogen in the genus *Fusarium* was found and only in two mature beetles (0.7%) in 2010. The pathogen species found during our study of *I. cembrae* were very similar to the pathogens previously identified for *Ips typographus*. No species-specific pathogen was detected.

Introduction

Of the seven species of bark beetles in the genus *Ips* that occur in Europe (http://www.faunaeur.org/), several attack multiple tree hosts (Pfeffer 1995). This probably explains why some of the pathogens of bark beetles also have multiple hosts. The pathogens of *Ips typographus* (Linnaeus, 1758) have been studied by numerous authors (Takov et al. 2010) but those of *Ips duplicatus* (Sahlberg, 1836), *Ips amitinus* (Eichhoff, 1871), *Ips sexdentatus* (Börner, 1776) and *Ips acuminatus* (Gyllenhal, 1827) have been studied only infrequently (Wegensteiner 2004; Weiser et al. 2006; Holuša et al. 2007, 2009; Takov et al. 2010). Pathogens of one species of bark beetle, *Ips cembrae* (Heer, 1836), have never been reported, even though this beetle can increase to high numbers (Takov et al. 2010).

Ips cembrae, whose common name is the large larch bark beetle, occurs in Europe (Austria, Croatia, Czech Republic, Denmark, England, Finland, France, Germany, Hungary, Italy, Netherlands, Poland, Romania, Scotland, Serbia and Montenegro, Slovakia, Slovenia, Sweden, Switzerland, Ukraine and Wales) and in central Russia (OEPP/EPPO 2005). *I. cembrae* has produced several local outbreaks throughout central Europe in recent years (Knížek and Zahradník 2004; Krehan and Cech 2004; Stratmann 2004; Knížek and Holuša 2007; Grodzki 2009). Even though the scale of sanitary wood cutting caused by *I. cembrae* infestation is small compared to that caused by other bark beetles in central Europe (Zahradník 1997), *I. cembrae* is considered a serious pest in some European countries (Grégoire and Evans 2004). It has sometimes damaged more than 100 m^3 of wood per locality (J. Holuša, unpublished observation).

The aim of this work was to determine the spectrum of species pathogenic to *I. cembrae* and to compare the pathogens of *I. cembrae* with those of other *Ips* species.

Materials and Methods

Sites and sampling methods

Ips cembrae beetles were collected from 2007 to 2011 at 10 study sites in central Europe (fig. 1). Beetles were collected from trap trees [*Larix decidua* (Mill.)], from pheromone traps near *L. decidua* trees, and from emergence traps after immature stages were reared in field-infested *L. decidua* logs in the laboratory. *I. cembrae* had an elevated population density at all study sites, and so all trap trees were abundantly infested. Table 1 summarizes the study sites, methods used and study years. Details concerning the methods used to obtain beetles are provided in the following paragraphs.

Fig. 1 Mean infection levels of *Chytridiopsis typographi* (represented in black in graphs) in maternal beetles of *Ips cembrae* in central Europe. Study sites are identified by numbered points (see table 1). Pie graphs are for trap trees, bar graphs are for pheromone traps, and square graphs are for sections of infested log that were incubated in the laboratory. Infection level is equivalent to the percentage of the graph area that is filled. For example, a graph that was half filled with black would indicate that 50% of the collected beetles were infected.

A set of three trap trees was prepared at each of the study sites in April (years are listed in table 1). The study sites were chosen on previous knowledge of outbreak populations of *I. cembrae*. These trap trees were used to capture overwintering beetles in April or to capture beetles of the new generation (offspring beetles) in June. Mature beetles were collected from gallery systems with larvae in the second or third instar, or as pupae or callow adult beetles. Beetles collected from the trap trees were placed in 2-ml Eppendorf micro-test tubes (one beetle per tube). A piece of wet gauze was added to the test tube to maintain 100% relative humidity. The beetles were immediately frozen and stored at −4°C.

Ips cembrae beetles were also collected using Theysohn® (Theysohn, Salzgitter, Germany) slot barrier traps with Cembräwit® (WITASEK Pflanzenschutz GmbH, Feldkirchen, Germany) pheromone evaporators at three of the study sites. Sets of five pheromone traps were placed 10–15 m in front of sunlit forest stand at the edge of a wooded glade. Lures were placed in the traps in mid-April, just before the beginning of emergence, and were renewed 8 weeks later. The traps were checked at 7- to 10-day intervals. Beetles were sampled in this manner until the end of September. The beetles collected from pheromone traps were stored as described in the previous paragraph.

For the third sampling method, beetle-infested log sections from trap trees from three of the study sites (table 1) were transported to the laboratory, where they were placed in emergence traps and incubated at 24°C with long-day light conditions (L : D = 16 : 8 h). Log sections were sprayed with water every second day. The hours of daylight and darkness were appropriate for the season in which the beetle-infested logs were taken. Logs from the Oberwölbling study site were moved into the laboratory on 10 June 2008, and those from the Rudziniec and Slezské Rudoltice study sites on 10 September 2008. Offspring beetles were sampled each day upon their emergence.

Examination of beetles

The beetles collected by the first two methods remained intact after being frozen and were dissected in a water drop within 4 months of collection. The beetles collected by the third methods were dissected when still alive. Dissection was performed by removing the entire gut together with parts of the

Table 1 Study sites in central Europe with methods, years of research and occurrence of pathogens

								Percentage of beetles with indicated pathogen				
Study site	Coordinates	Altitude (a.s.l.)	Method	Beetles	n	Year	N	*Chytridiopsis typographi*	*Gregarina typographi*	*Mattesia schwenkei*	*Nosema* sp.	*Fusarium* sp.
Oberwolbling (A)[1]	48°19′02″N, 15°34′09″E	430	LAB	OB	1	2008	143	36.4	2.1	0	0	0
Oberwolbling (A)[1]	48°19′02″N, 15°34′09″E	430	LAB	MB	1	2008	96	58.3	0	0	0	0
Rudziniec (PL)[7]	50°23′26″N, 18°24′42″E	204	LAB	OB	3	2008	112	6.3	0	0	0	0
Slezske Rudoltice (CZ)[3]	50°12′52″N, 17°40′56″E	275	LAB	OB	20	2008	505	18.4	0	0	0	0
Sokolovac (HR)[2]	46°10′37″N, 16°48′12″E	118	PT	MB	1	2011	160	10.0	1.2	0.6	0	0
Slezske Rudoltice (CZ)[3]	50°12′52″N, 17°40′56″E	275	PT	MB	1	2007	58	29.3	0	0	1.7	0
Slezske Rudoltice (CZ)[3]	50°12′52″N, 17°40′56″E	275	PT	MB	10	2008	289	21.2	0	0	0	0
Trzebina (PL)[4]	50°17′55″N, 17°36′03″E	260	PT	MB	1	2007	40	47.5	0	0	0	0
Hlubocec (CZ)[5]	49°50′30″N, 17°58′11″E	441	TT	MB	2	2009	105	11.7	0	0	0	0
Hlubocec (CZ)[5]	49°50′30″N, 17°58′11″E	441	TT	MB	2	2010	98	15.3	0	0	0	0
Oberwolbling (A)[1]	48°19′02″N, 15°34′09″E	430	TT	MB	1	2008	66	47.0	0	0	0	0
Pusta Polom (CZ)[6]	49°51′01″N, 17°59′50″E	434	TT	MB	2	2010	274	6.6	0	0	0	0.7
Rudziniec (PL)[7]	50°23′26″N, 18°24′42″E	204	TT	MB	1	2008	80	20.2	0	0	0	0
Slezske Rudoltice (CZ)[3]	50°12′52″N, 17°40′56″E	275	TT	MB	1	2008	47	2.1	0	0	0	0
Sumbark (CZ)[8]	49°47′60″N, 18°24′41″E	260	TT	MB	3	2009	183	12.5	0	0	0	0
Sumbark (CZ)[8]	49°47′60″N, 18°24′41″E	260	TT	MB	3	2010	326	2.2	0	0	0	0
Trzebina (PL)[4]	50°17′55″N, 17°36′03″E	260	TT	MB	1	2007	46	21.7	0	0	2.2	0
Trzebina (PL)[4]	50°17′55″N, 17°36′03″E	260	TT	OB	1	2007	30	0	0	0	0	0
Utechov (CZ)[9]	49°17′18″N, 16°37′59″E	450	TT	MB	3	2009	695	28.7	0	0	0	0
Velky Kosir (CZ)[10]	49°34′33″N, 17°04′34″E	442	TT	MB	1	2008	26	15.4	0	0	0	0

Superscript numbers refer to sites shown in fig. 1.

Beetles were obtained from infested log sections incubated in the laboratory (LAB); from pheromone traps (PT) in the field; or from trap trees (TT) in the field.

OB, offspring beetles; MB, maternal beetles; n, number of samples; N, number of beetles.

muscles, the fat body, Malpighian tubules and ovaries or testes. Fresh smears were examined with a light microscope [Reichert Polyvar (Reichert Inc., Depew, NY 14043) or Arsenal LPE 5013i-T (ARSENAL Co. Ltd., Praha, Czech Republic)] at 100 to 400× magnification. Smears were then air dried, fixed with methanol, stained with Giemsa dye and then re-examined at magnification 1000× (method described by Wegensteiner et al. 1996).

Results

Our examination of 3379 *I. cembrae* beetles resulted in the detection of five pathogen species (table 1). We found two microsporidian pathogens: a diplokaryotic microsporidium (probably *Nosema* sp.) and *Chytridiopsis typographi* (Weiser 1954) (fig. 2), and two gregarines: *Gregarina typographi* (Fuchs, 1915) and *Mattesia schwenkei* (Purrini, 1977). With respect to the fungal pathogen, a *Fusarium* sp. was recorded in the haemolymph of two mature beetles collected in one sample from the Czech Republic in 2010. This represented 0.8% of the beetles sampled in the Czech Republic in that locality (table 1).

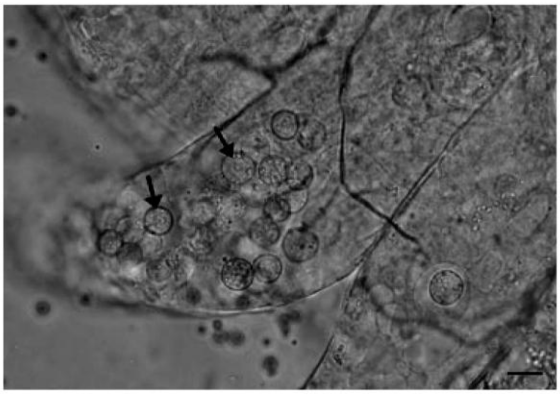

Fig. 2 *Chytridiopsis typographi* thick-walled cysts (arrows) in midgut of *Ips cembrae* obtained from logs in Oberwölbling (Austria). Bar 20 μm. (photo R. Wegensteiner).

The microsporidian pathogen *Nosema* sp. was found in the fat bodies of only two mature beetles from two study sites (table 1). *C. typographi*, in contrast, was found in all samples of mature beetles (table 1 and fig. 1), with a mean infection level of 16.7%. The infection level of *C. typographi* ranged from 0 to 58% in mature and offspring beetles.

We determined the average size of the thick-walled cysts (n = 170) of *C. typographi* in the midgut epithelium of beetles collected in 2010 (n = 17). Ten cysts were measured in each infected *I. cembrae* individual. The measured values were normally distributed (Shapiro–Wilk test, W = 0.99, P > 0.05) and the cyst diameter averaged 20 ± 3.4 μm (fig. 3).

Gregarina typographi was recorded in 1–2% of the beetles at only two study sites (table 1). Trophozoites and gamonts of gregarines were never observed in the stomodeum but always in the midgut lumen. *M. schwenkei* was observed in the fat body of one beetle (0.6% of beetles in that sample) from Croatia (table 1).

Discussion

Ips cembrae is a secondary pest of the larch (*Larix decidua*) across the entire area of this tree's distribution, from the lowest altitudes to the subalpine zone (Postner 1974). *I. cembrae* occasionally colonizes spruce *Picea abies* (L.) Karsten (Pfeffer 1989). A reported infestation on pine (*Pinus cembra* L.) has been revised [*Ips amitinus* (Eichhoff, 1871) had incorrectly been identified as *I. cembrae*] (Pfeffer 1995).

The list of microsporidian, protozoan and viral pathogens known from bark beetles of the genus *Ips* includes nine species (Wegensteiner 2004; Takov et al. 2010). Some of these can infect more than one bark beetle species (Haidler 1998; Händel et al. 2001, 2003). Its preference for a narrow range of host trees reduces the contact between *I. cembrae* and other species of bark beetles and other pathogens as well. On the other hand, *I. typographus* and *I. amitinus* do occur occasionally on *Pinus* spp. (Pfeffer 1995). The pathogen species found during our study of *I. cembrae* were similar to the pathogens known from *I. typographus* if one excludes endemic species of pathogens.

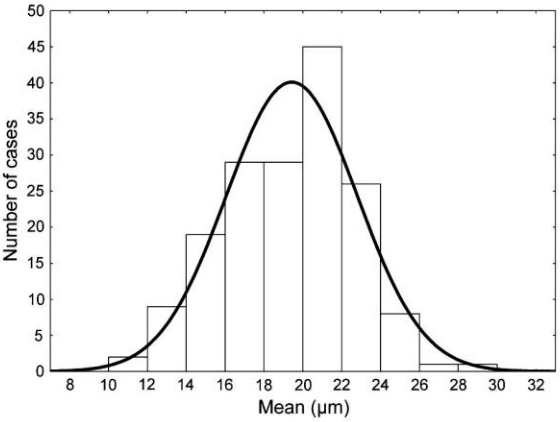

Fig. 3 Diameters (n = 170) of *Chytridiopsis typographi* cysts (thick-walled) in the midgut of *Ips cembrae*. The line shows the normal distribution.

The detected *Nosema* sp. is likely to *Nosema typographi* (Weiser 1954), a species commonly occurring in beetles of the genus *Ips*, but with a low prevalence (Weiser 1955; Wegensteiner and Weiser 1996; Händel et al. 2003; Pultar and Weiser 2004) because infection occurs in the fat body (Purrini 1978; Wegensteiner and Weiser 1996), and its transmission is therefore complicated. Among the group of pathogens infecting adipose tissue, transmission typically occurs after the death and decomposition of the host (Weiser et al. 2000). Only in the case of intensive infection would *N. typographi* attack the ovaries and then transovarial transmission could occur (Weiser et al. 2000), as is the case for other species of microsporidia in invertebrates (Phelps and Goodwin 2008).

Chytridiopsis typographi, a common pathogen of the genus *Ips* (Wegensteiner 2004; Takov et al. 2010), was detected by all methods and at all study sites. The infection level of this pathogen was usually low but was >45% in three cases. *I. cembrae* is a newly recognized host for this microsporidian species. The thick-walled cysts of *C. typographi* in *I. cembrae* are nearly identical to those of *C. typographi* in *I. typographus*, in which they typically range from 10 to 20 μm in diameter (Weiser 1954, 1970). The mechanism of microsporidian spore germination can result in two routes of infection. Most often, the beetles are infected horizontally by spores that inhabit the environment and especially by spores in faeces within the gallery system. *C. typographi* can also infect vertically (transovarially), and because the infection was similar among offspring beetles and mature parental beetles, infection in the current study apparently resulted from vertical transmission.

Gregarina typographi is a quite common and non-specific pathogen of bark beetles. It has been found in the midgut lumen in a number of members of the Scolytinae subfamily (Wegensteiner 2004; Kereselidze et al. 2010; Takov et al. 2010). About 53% of bark beetle species are hosts to *G. typographi*

(Takov et al. 2010), and in some cases, the prevalence in a population can be high (up to 44%) (Wegensteiner et al. 1996; Wegensteiner 2004). Although *C. typographi* and *G. typographi* often occur together with high infection levels (Haidler et al. 2003), a high infection level is also possible when only one pathogen is present (Kereselidze and Wegensteiner 2007).

The following two pathogens that were previously recorded in *I. typographus* in the Šumava Mountains (Pultar and Weiser 2004) and in Austria (Wegensteiner 1994; Wegensteiner et al. 1996; Händel et al. 2001, 2003) were absent in our samples: *Entomopoxvirus typographi* (*It*EPV) (Wegensteiner and Weiser, 1995) and *Malamoeba scolyti* (Purrini, 1980). The microsporidium *Unikaryon montanum* (Weiser, Wegensteiner and Žižka, 1998), known from locations in Austria and Germany (Weiser et al. 1998; Wegensteiner and Weiser 2004), was also not found in our study. The neogregarine *Menzbieria chalcographi* (Weiser 1955) was not found, but this species is generally rare even though its occurrence can be very high in some cases (Pultar and Weiser 2004; Kereselidze and Wegensteiner 2007).

Larssoniella duplicati Weiser, Holuša et Žižka, 2006, which was not found in the current study, is definitely specific to *I. duplicatus* (Weiser et al. 2006; Holuša et al. 2007, 2009). *L. duplicati* can achieve a high infection level in *I. duplicatus* (Holuša et al. 2007, 2009), and a high infection level frequently occurs throughout the beetle's range (Holuša et al. 2007).

Differences in infection levels caused by horizontal transmission may be related to the date when the beetles were collected because this affects the length of time that beetles remain together in nuptial chambers. *G. typographi* infection levels, for example, increase with the time that beetles remain in the same galleries increases (Lukášová and Holuša 2011). Because gregarines develop rapidly, horizontal transmission of infection among parental beetles inhabiting a single nuptial chamber could be significant (see also MacDougall 1942; Smith et al. 2007).

The pathogens found in our study of *I. cembrae* were very similar to the pathogens known from *I. typographus* and other species in the genus *Ips*. A total of about 10 pathogens are known from these hosts, three of which are very common (Wegensteiner and Weiser 2004; Kereselidze et al. 2010; Takov et al. 2011). Among the common pathogens, two were found (*G. typographi* and *C. typographi*) in *I. cembrae* but one (*It*EPV) was not even in outbreak populations. It was interesting to find *Ch. typographi* in beetles from all geographical regions, *G. typographi* in beetles from Austria and Croatia, *Nosema* sp. in beetles from Czech Republic and Poland. In addition, pathogen species diversity was highest in beetles from Croatia. No species-specific pathogen was detected. Given the substantial range of the study sites, we suspect that species-specific pathogens of the large larch beetle, *I. cembrae*, do not exist or are rare.

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References

Grégoire JC, Evans HF, 2004. Damage and control of BAWBILT organisms – an overview. In: Bark and wood boring insects in living trees in Europe, a synthesis. Eds. by Lieutier F, Day KR, Battisti A, Grégoire JC, Evans HF, Kluwer Academic, Dordrecht, 19–37.

Grodzki W, 2009. Kornik modrzewiowiec *Ips cembrae* (Heer) (Coleoptera, Curculionidae, Scolytinae) w drzewostanach modrzewiowych południowej Polski. Leś. Prac. Bad. 70, 355–361.

Haidler B, 1998. Pathogene und Parasiten von Fichtenborkenkäfern in einem Fichtenbestand am Achner Kogel bei Tamsweg. Diploma thesis. Formal- und Naturwissenschaftliche Fakultät der Universität Wien, Wien, 144.

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. Insect pathogens and insect parasitic nematodes. IOBC WPRS Bull. 26, 257–260.

Händel U, Kenis M, Wegensteiner R, 2001. Untersuchungen zum Vorkommen von Pathogenen und Parasiten in Populationen überwinternder Fichtenborkenkäfer (Col., Scolytidae). Mitt. Dtsch. Ges. Allg. Angew. Entomol. 13, 423–428.

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. J. Pest Sci. 76, 22–32.

Holuša J, Weiser J, Drápela K, 2007. Pathogens of *Ips duplicatus* (Coleoptera, Scolytidae) in three areas in Central Europe. Acta Protozool. 46, 157–167.

Holuša J, Weiser J, Žižka Z, 2009. Pathogens of the spruce bark beetles *Ips typographus* and *Ips duplicatus*. Cent. Eur. J. Biol. 4, 567–573.

Kereselidze M, Wegensteiner R, 2007. Occurrence of pathogens and parasites in *Ips typographus* L. from spruce stands (*Picea orientalis* L.) in Georgia. IOBC WPRS Bull. 30, 207–210.

Kereselidze M, Wegensteiner R, Goginashvili N, Tvaradze M, Pilarska D, 2010. Further studies on the occurrence of natural enemies of *Ips typographus* (Coleoptera: Curculionidae: Scolytinae) in Georgia. Acta Zool. Bulg. 62, 131–139.

Knížek M, Holuša J, 2007. Podkorní hmyz. In: Výskyt lesních škodlivých činitelů v roce 2006 a jejich očekávaný stav v roce 2007. Ed. by Knížek M, Zprav. Ochr. Lesa Suppl., VÚLHM, v.v.i. 74, 21–32.

Knížek M, Zahradník P, 2004. Podkorní hmyz. In: Výskyt lesních škodlivých činitelů v roce 2003 a jejich očekávaný stav v roce 2004. Eds. by Kapitola P, Knížek M, Baňař P, Zprav. Ochr. Lesa Suppl., VÚLHM Jíloviště – Strnady, 80, 30–39.

Krehan H, Cech TL, 2004. Larch damage in Upper Styria. An example of the complex effects of damage agents. Forstsch. Aktuell 32, 4–8.

Lukášová K, Holuša J, 2011. *Gregarina typographi* (Eugregarinorida: Gregarinidae) in th bark beetle *Ips typographus* (Coleoptera: Curculionidae): changes of infection level in breeding system. Acta Protozool. 50, 311–318.

MacDougall MM, 1942. A study of temperature effects on gregarines of *Tenebrio molitor* larvae. J. Parasitol. 28, 233–240.

OEPP/EPPO, 2005. *Ips cembrae* and *Ips subelongatus*. Bull. OEPP/EPPO 35, 445–449.

Pfeffer A, 1989. Kůrovcovití Scolytidae a jádrohlodovití Platypodidae. Academia, Praha.

Pfeffer A, 1995. Zentral- und westpaläarktische Borken- und Kernkäfer (Coleoptera: Scolytidae, Platypodidae). Pro Entomologia, Basel.

Phelps NBD, Goodwin AE, 2008. Vertical transmission of *Ovipleistophora ovariae* (Microspora) within the eggs of the Golden Shiner. J. Aquat. Anim. Health 20, 45–53.

Postner M, 1974. *Ips cembrae*. In Die Forstschädlinge Europas. II. Band. Käfer. Ed. Schwenke W, Paul Parey, Hamburg, 458–459.

Pultar O, Weiser J, 2004. Výsledky posledních patologických studií dominantních kůrovců v NP Šumava a jejich využití (Results of the last pathological studies of dominant bark beetles in the National park Šumava and their using in biocontrol). In: Sborník referátů. 28. setkaní lesníků tří generací na téma ''Nebezpečí kůrovce v roce 2004, 19.3.2004 (Praha-Novotného lávka), Česká lesnická společnost, VÚLHM Jíloviště-Strnady, Lesn. práce.

Purrini K, 1978. Protozoen als Krankheitserreger bei einigen Borkenkäferarten (Col., Scolytidae) im Königsee-Gebiet, Oberbayern. Anz. Schädl., Pflanz., Umwelt. 51, 171–175.

Smith AJ, Cook TJ, Lutterschmidt WI, 2007. Effects of temperature on the development of *Gregarina cubensis* (Apicomplexa: Eugregarinida) parasitizing *Blaberus discoidalis* (Blattaria: Blaberidae). J. Parasitol. 93, 583–588.

Stratmann J, 2004. Borkenkäferkalamität 2003, was haben wir gelernt, sind wir für 2004 gerüstet? Forst und Holz 59, 166–169.

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 Zoolog. Bulg. 62, 201–209.

Takov D, Doychev D, Linde A, Draganova S, Pilarska D, 2011. Pathogens of bark beetles (Coleoptera: Curculionidae) in Bulgarian forests. Phytoparasitica 39, 343–352.

Wegensteiner R, 1994. *Chytridiopsis typographi* (Protozoa, Microsporidia) and other pathogens in *Ips typographus* (Coleoptera, Scolytidae). IOBC WPRS Bull. 17, 39–42.

Wegensteiner R, 2004. Pathogens in bark beetles. In: Bark and wood boring insects in living trees in Europe, a synthesis. Eds. by Lieutier F, Day KR, Battisti A, Grégoire JC, Evans HF, Kluwer, Dordrecht, 291–313.

Wegensteiner R, Weiser J, 1996. Untersuchungen zum Auftreten von Pathogenen bei *Ips typographus* (Col., Scol.) aus einem Naturschutzgebiet im Schwarzwald (Baden-Württemberg). Anz. Schädl., Pflanz., Umwelt. 69, 162–167.

Wegensteiner R, Weiser J, 2004. Annual variation of pathogen occurrence and pathogen prevalence in *Ips typographus* L (Col, Scolytidae) from the BOKU University Forest Demonstration Centre. J. Pest. Sci. 77, 221–228.

Wegensteiner R, Weiser J, Führer E, 1996. Observations on the occurrence of pathogens in the bark beetle *Ips typographus* L. (Col.,Scolytidae). J. Appl. Entomol. 120, 190–204.

Weiser J, 1954. Příspěvek k znalosti cizopasníků kůrovce *Ips typographus* L. Věst. Česl. Spol. Zool. 18, 217–224.

Weiser J, 1955. Příspěvek k znalosti cizopasníků kůrovce *Ips typographus* L II (Contributions to the knowledge of *Ips typographus* parasites, II.). Věst. Česl. Spol. Zool., 19, 374–380.

Weiser J, 1970. Three new pathogens of the douglas fir beetle *Dendroctonus pseudotsugae*: *Nosema dendroctoni* n.sp., *Ophryocystis dendroctoni* n.sp., and *Chytridiopsis typographi* n.comb. J. Invertebr. Pathol. 16, 436–441.

Weiser J, Wegensteiner R, Žižka Z, 1998. *Unikaryon montanum* sp. n (Protista, Microspora), a new pathogen of the spruce bark beetle, *Ips typographus* (Coleoptera, Scolytidae). Fol. Parasitol. 45, 191–195.

Weiser J, Pultar O, Žižka Z, 2000. Biological protection of forest against bark beetle outbreaks with poxvirus and other pathogens. IUAPPA Section B, 12, 168–172.
Weiser J, Holuša J, Žižka Z, 2006. *Larssoniella duplicati* n.sp. (Microsporidia, Unikaryonidae) in the double – spined spruce bark beetle, Ips duplicatus (Coleoptera, Scolytidae) in Central Europe. J. Pest. Sci. 79, 127–135.
Zahradník P, 1997. Výskyt lesních škodlivých činitelů v roce 1996 a jejich očekávaný stav v roce 1997. Zprav. Ochr. Lesa. Suppl. VÚLHM Jíloviště-Strnady 4, 1–8.

4.6 *Gregarina typographi* (Eugregarinorida: Gregarinidae) in the bark beetle *Ips typographus* (Coleoptera: Curculionidae): changes in infection level in the breeding system

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Gregarina typographi (Eugregarinorida: Gregarinidae) in the Bark Beetle *Ips typographus* (Coleoptera: Curculionidae): Changes in Infection Level in the Breeding System

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Summary. Changes in *Gregarina typographi* Fuchs, 1915 (Eugregarinorida: Gregarinidae) infection levels were studied in a population of the bark beetle *Ips typographus* (L., 1758) (Coleoptera: Curculionidae), which lives in association with Norway spruce. Beetles were repeatedly collected from three logs of trap trees during 2009 and 2010 at one study site in the Czech Republic with a high level of *G. typographi* infection (seasonal mean of 15.7% in 2009 and 19.8% in 2010). Infection levels did not differ statistically between sexes, logs, and trap trees but did differ among sampling dates. During the beetle reproductive period, the infection levels nearly doubled in 2009 (from 10.7 to 19.8%) and more than tripled in 2010 (from 9.3 to 31.3%). We infer that the continuous increase in the *G. typographi* infection level within each of the two years resulted from transmission among beetles in nuptial chambers during the May–June reproductive and egg-laying period.

Key words: Beskids, Gregarinidae, horizontal transmission, protozoan disease, spruce bark beetle.

INTRODUCTION

Several authors (Wegensteiner 2004, Takov *et al.* 2010, Wegensteiner *et al.* 2010) have studied pathogens in *Ips typographus* (L., 1758). The spruce bark beetle *I. typographus* is the most damaging insect attacking spruce forests (*Picea abies* (L., 1753) Karsten, 1881) in Eurasia (Christiansen and Bakke 1988). Adult beetles of *I. typographus* emerge from the forest litter and tree bark on warm spring days and fly to stressed host trees. These bark beetles use an aggregation pheromone to attract more individuals of the same species to the tree for the purpose of weakening the tree and mating. The pheromone attracts both sexes. The attracted males join the attack and secure an area for mating and oviposition. This area consists of a hole and a chamber beneath the bark known as a "nuptial chamber". The females construct a tunnel ("maternal gallery") beneath the nuptial chamber in which to lay eggs. In all species of the *Ips* genus, several females (usually two or three) join each male in his nuptial chamber (Wermelinger 2004). At lower elevations, overwintering *I. ty-*

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pographus beetles emerge in April/May and produce one or two generations of offspring per year with the main peaks of bark beetle emergence in July and August/September. At elevations above 1,000 m a.s.l., this species produces only one generation of offspring per year (Wermelinger 2004). The emergence and migration of beetles of monovoltine generations depend on the geographical latitude: northern populations emerge later and fewer beetles move before overwintering than in southern populations (Forsse 1991).

Gregarina typographi Fuchs, 1915 has been found in the midgut lumen in a number of members of the Scolytinae subfamily (Wegensteiner 2004, Kereselidze *et al.* 2010, Takov *et al.* 2010). Insects usually are infected when they ingest oocysts contained in host faeces or in dead or living (in the case of cannibalism) body parts of the host. Most of authors (Wegensteiner 2004, Takov *et al.* 2010) monitored levels of infection in the populations; only Wegensteiner *et al.* (2010) tried to determine the impact of *G. typographi* on the demographic structure of *I. typographus*. Because gregarines develop rapidly, horizontal transmission of infection among parental beetles inhabiting a single nuptial chamber could be significant (MacDougall 1942, Smith *et al.* 2007). Each male beetle possesses a harem that usually consists of 2–3 females, and the male and females inhabit a gallery system (nuptial chamber) for several weeks (Wermelinger 2004). In this study, we observed changes in the infection level of *G. typographi* in *I. typographus* in the nuptial chamber during two seasons from the time when beetles begin burrowing into the trees until the end of the egg-laying period.

MATERIALS AND METHODS

Study site

Gregarina typographi and *I. typographus* were studied at the Smrk Nature Reserve in the Beskids (Czech Republic). The Beskids bioregion has a terrain characterized by mountains with typical elevations of 500–1,200 m a.s.l. Forest cover exceeds 70%, and more than 70% of that cover is represented by spruce (Culek 1996). The old-growth forest at the Smrk Nature Reserve consists of beech and spruce trees. The study site was situated in the center of the reserve (coordinates: 49°30′N, 18°22′E) within an elevation range of 1,180 to 1,200 m a.s.l. In 2009, the average temperature varied 4–5°C, and the total precipitation was about 1,400 mm (www.chmu.cz).

Spruce bark temperature was calculated from average monthly air temperature and global radiation (obtained from www.chmu.cz) during the study period in each year according to Baier *et al.* (2007). The mean bark temperature in 2009 was around 7.7°C in May and 10.7°C in June and was slightly higher the following year at around 11.8°C in June and 15.3°C in July.

The Reserve is a protected area with only minimal forestry management, represented by limited sanitation felling, trap-tree importation and removal, and beetle trapping in pheromone traps.

Trapping of beetles

The study involved 11 trap trees (*Picea abies*) in 2009 and 5 trap trees (*P. abies*) in 2010. Trap trees were imported from neighboring managed forests. Each trap tree (about 30 cm in diameter, 30 m in height) was cut into three logs of the same length (log I–III), which were situated in parallel with 1–2 m spacing in the center of the nature reserve. The logs were located in a clearing perpendicular to a trail through the forest, and the logs from each tree were ca. 50 m distant from the logs of the other trees. Trap trees were placed at the site on 12 May 2009 and 13 May 2010. Mature beetles were collected under a strip of bark from each log of trap tree during 2009 (26 May, 18 June) and 2010 (at 10-day intervals in June and July). Each sample area was 1 m long and about 0.5 m wide (half the tree circumference). The samples collected on different dates were separated by 1 m.

All mature beetles in nuptial chambers and maternal galleries beneath each strip of bark were collected. Analyzed beetles from each sample date and bark strip were always chosen at the same stage of development of offspring in the breeding system (from nuptial chambers to callow beetles). Thus only beetles from the same period of infestation, i.e. at the end of May (for 2009) and in early June (in 2010), were chosen. The entry holes were counted, and the individual beetles were placed into 2-cm^3 Eppendorf micro test tubes. A piece of wet gauze was added to the test tube to maintain 100% relative humidity. The beetles were immediately frozen and stored at –4°C. The tissues of beetles thus prepared remained intact. The entire body cavity (including intestines, Malpighian tubules, adipose tissue and gonads) were dissected in a water drop within 4 months of collection and checked for pathogens.

During the last analysis of beetles (20 July 2010), we collected a samples of frass and faeces from maternal galleries (length 60–80 mm) and entry holes. Frass was removed with a brush and crushed into drops of water. The final suspension was examined under light microscope and checked for presence of gregarine oocysts.

Determination of infection and statistical analysis

In 2009 and 2010, each dissected beetle was examined with a light microscope at magnifications of 40–400 × to determine its sex and whether it was infected with *G. typographi* or any other pathogen. For beetles collected in 2010, life stages of *G. typographi* were measured and photographed with an Arsenal LPE 5013i-T microscope (Arsenal s.r.o., Prague, Czech Republic). When trophozoites, gamonts and gametocysts occurred together, we recorded all these stages and the data were included (individually) in the analysis of individual life stages. To compare the total prevalence of *G. typographi*, we used only the presence/absence of a pathogen (all stages combined). The following structures of trophozoites and gamonts were measured: length of deutomerite (LD), length of protomerite (LP), total length (TL), width of deutomerite (WD), width of protomerite (WP), ratio of the length of protomerite to total length (LP : TL), ratio of the width of protomerite to the width of deutomerite (WP : WD), and ratio of the width of protomerite to the length of protomerite (WP : LP).

Infections were compared by year (Kruskal-Wallis test), collection date (one-way ANOVA), sex of the beetle (Wilcoxon rank-sum test), and trap tree (one-way ANOVA), such that infections of all beetles collected from a trap tree (> 100 beetles) were calculated. For comparison of infections among the individual trap trees (one-way ANOVA), data from beetles collected from a sample were used (> 20 beetles). The R 2.10.1 software program and STATISTICA 9 were used for statistical analyses.

RESULTS

Gregarina typographi morphometrics and life stages

G. typographi gametocysts were spherical with a diameter of 93.6 ± 20.2 µm (n = 30) and were observed only in the proctodeum. The dimensions of gregarine trophozoites (Fig. 1) and gamonts are reported in Table 1. Both stages were always found from the stomodeum to midgut.

In 2010, the presence of *G. typographi* life cycle stages in different samples were recorded (Fig. 2). The percentage of beetles with gamonts (Fig. 2B), and gametocysts (Fig. 2C) did not differ among the sampling times. The percentage of beetles with trophozoites, however, tended to increase over time and was 3.45 times greater at the last sampling date than at the first sampling date (Kruskal-Wallis test; chi-square = 9.50, $p < 0.05$) (Fig. 2A).

Infection rates

The mean infection rate of *G. typographi* in *I. typographus* was 15.7% in 2009 and 19.8% in 2010. *G. typographi* infection levels did not significantly differ between the individual years (Kruskal-Wallis test; chi-square = 1.00, $p > 0.05$).

For 2009, a total of 1,624 *I. typographus* beetles were dissected. Data for infection levels for individual trap trees in 2009 were normally distributed (Shapiro-Wilk test for normality; W = 0.97, $p > 0.05$). Infection levels differed significantly among the sampling dates in 2009 (ANOVA; F = 5.69, $p < 0.05$) (Fig. 3) but did not differ among trap trees or logs. *G. typographi* infection averaged 10.7% in May 2009 (n = 792 beetles) and 19.8% in June 2009 (n = 454 beetles). The only other pathogen detected in 2009 was a single microsporidium of *Chytridiopsis typographi* (Weiser 1954) Weiser 1970. *G. typographi* infection levels for females (16.1%) and males (15.9%) did not differ in 2009 (Wilcoxon rank-sum test; W = 2638.5, $p > 0.05$). In 2009, there were 0.6 entry holes of *I. typographus* per dm^2 of trap tree in June.

In 2010, a total of 822 *I. typographus* beetles were dissected. Data for infection levels for individual trap trees in 2010 were normally distributed (Shapiro-Wilk test for normality; W = 0.94, $p > 0.05$), and therefore one-way ANOVA (F = 3.57, $p < 0.05$) was used. Mean values of *G. typographi* infection did not differ among trap trees (ANOVA; F = 3.41, $p > 0.05$). According to multivariate analysis, the infection rate was significantly lower on 10 June 2010 than on 1 July and 10 July 2010 (ANOVA; F = 5.33, $p < 0.05$) (Fig. 4). More males (28.3%) than females (16.8%) were infected, but this difference was not statistically significant (Wilcoxon rank-sum test; W = 147.5, $p > 0.05$). No other pathogen was found in any organ or haemolymph in

Table 1. Morphometrics of trophozoites (n = 30) and gamonts (n = 30) of *Gregarina typographi* in *Ips typographus*. Measurements are in µm.

Stage		TL	LP	LD	WP	WD	LP : TL	WP : WD	WP : LP
Trophozoites	Min	103.5	20.1	55.0	7.3	37.2	0.2	0.1	0.2
	Max	277.2	52.1	186.3	29.7	148.4	0.3	0.4	1.0
	Mean	163.0	35.1	109.1	16.6	79.7	0.2	0.2	0.5
	SD	53.5	9.7	45.9	6.7	28.1	0.1	0.1	0.2
Gamonts	Min	105.0	16.1	82.1	31.4	49.2	0.2	0.4	0.8
	Max	241.5	99.7	182.6	82.8	143.5	0.4	0.7	2.2
	Mean	186.1	48.4	130.7	60.2	107.3	0.3	0.6	1.4
	SD	40.8	22.4	27.6	14.5	27.6	0.1	0.1	0.5

TL – total length; LP – length of protomerite; LD – length of deutomerite; WP – width of protomerite; WD – width of deutomerite; LP : TL – the length of protomerite to total length; WP : WD – the width of protomerite to the width of deutomerite; WP : LP – the width of protomerite to the length of protomerite; Min – minimum value; Max – maximum value; SD – standard deviation.

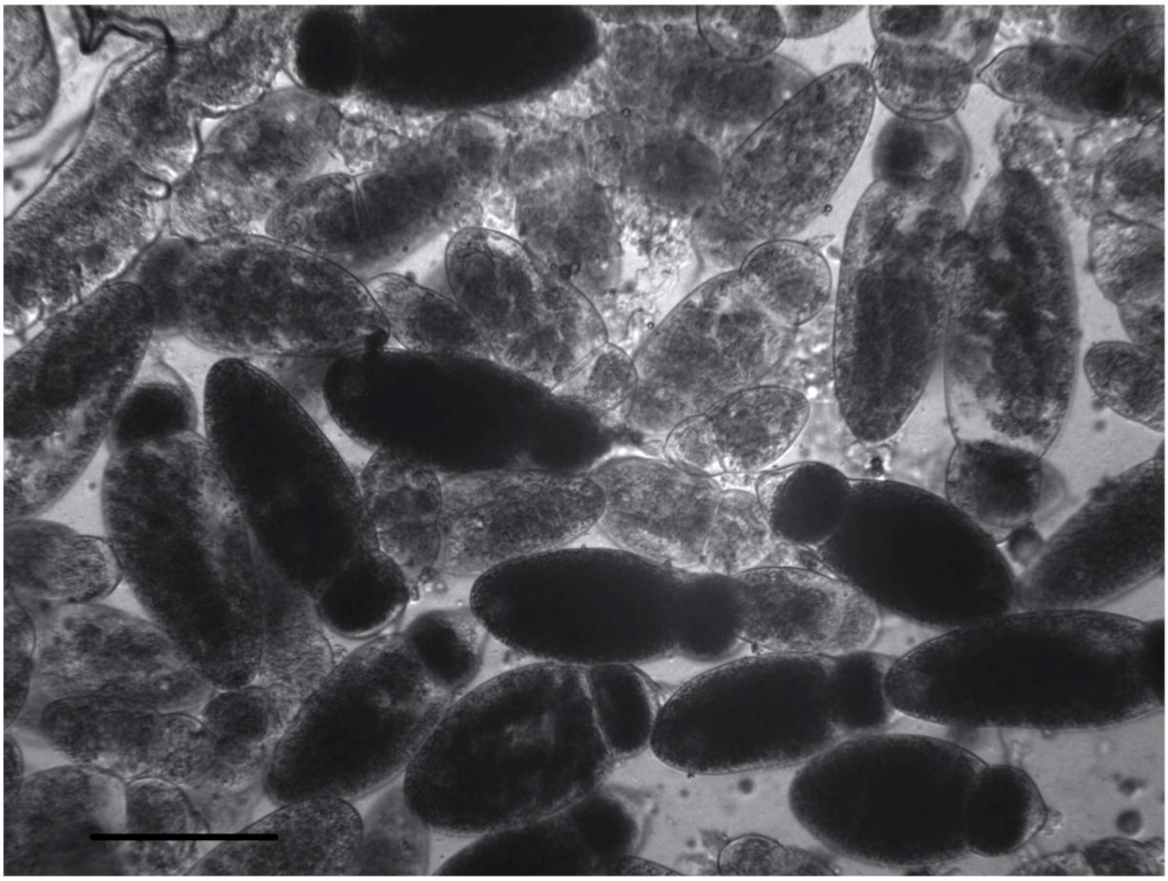


Fig. 1. *Gregarina typographi* trophozoites outside the body cavity of *Ips typographus* obtained from trap trees in 2010. Bar: 100 µm.

▸▸

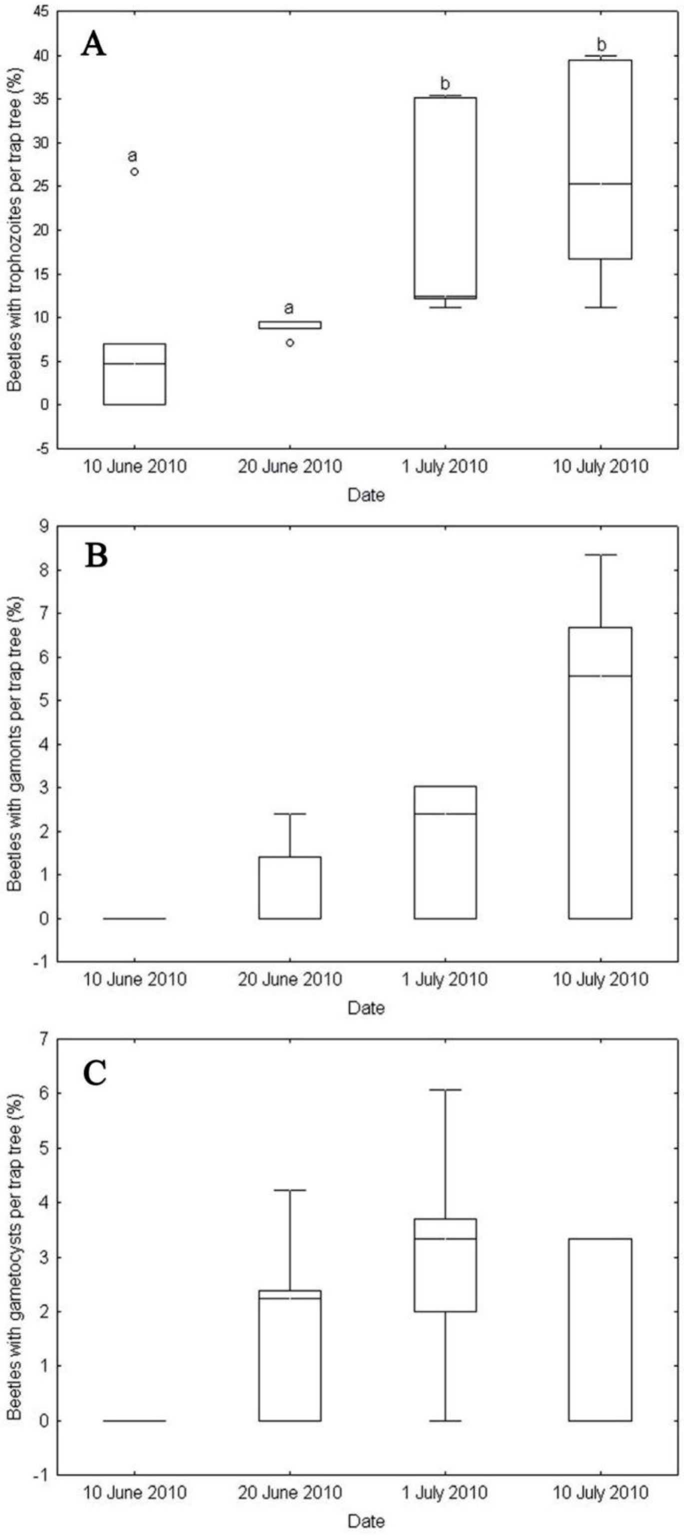
Fig. 2. Percentage of *Ips typographus* infected by *Gregarina typographi* in 2010 by stage: **(A)** trophozoites, **(B)** gamonts, and **(C)** gametocysts. Boxplots show median plus upper and lower quartiles. Minimum and maximum values are shown by the upper and lower whiskers (1.5 × interquartile range), and outlying values are depicted as circles. In the trophozoites graph (A), different letters above each bar indicate a significant difference between mean values (Kruskal-Wallis test, $p < 0.05$).

2010. The average population density on one trap tree was 0.4 entry holes of *I. typographus* per dm^2.

No gregarine oocysts were found in host frass and faeces from maternal galleries (n = 50) and entry holes (n = 20).

DISCUSSION

Gregarina typographi is the only representative of eugregarines found in the genus *Ips* (Wegensteiner 2004). From *Ips acuminatus* (Gyllenhal 1827) is far closer blank recorded species of the genus *Gregarina* sp. (Takov *et al.* 2007). Based on the morphology and morphometrics reported by other authors (Yaman 2007, Takov *et al.* 2007) for the individual stages of *Gregarina*, it is certain that the species observed in this study was also *G. typographi*. Precise detection of this parasite is problematic, because intracellular stages (sporozoites) of the eugregarine life cycle are difficult to identify with a light microscope (Bjørnson 2008, Yaman 2008). This could result in a slight underestimation


A
Beetles with trophozoites per trap tree (%)
45
40
35
30
25
20
15
10
5
0
-5
a
a
b
b
10 June 2010
20 June 2010
1 July 2010
10 July 2010
Date
B
Beetles with gamonts per trap tree (%)
9
8
7
6
5
4
3
2
1
0
-1
10 June 2010
20 June 2010
1 July 2010
10 July 2010
Date
C
Beetles with gametocysts per trap tree (%)
7
6
5
4
3
2
1
0
-1
10 June 2010
20 June 2010
1 July 2010
10 July 2010
Date

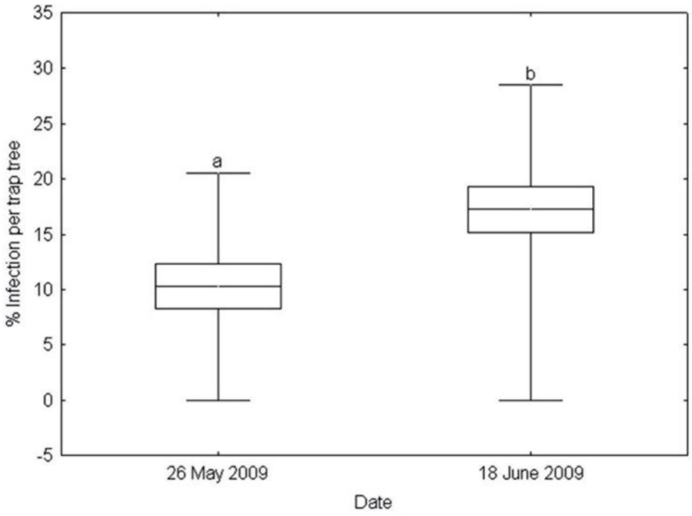


Fig. 3. Percentage of *Ips typographus* infected by *Gregarina typographi* per trap tree in 2009. Boxplots show mean ± standard deviation. Minimum and maximum values are shown by the upper and lower whiskers. Different letters above each bar indicate a significant difference between mean values (ANOVA, $p < 0.05$).

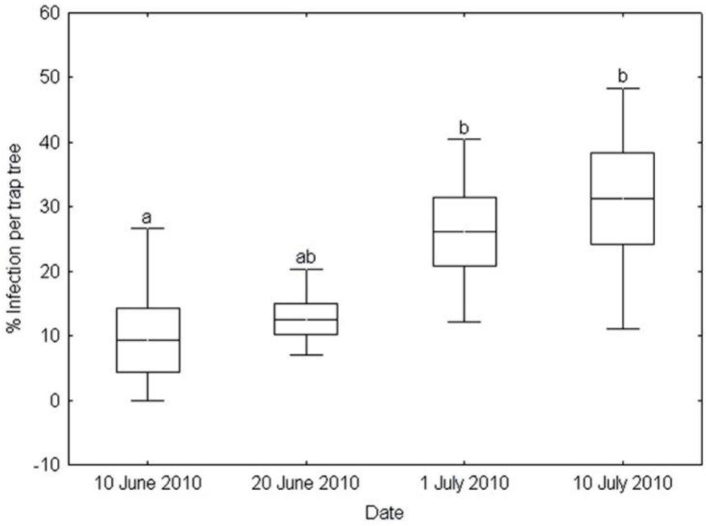


Fig. 4. Percentage of *Ips typographus* infected by *Gregarina typographi* per trap tree in 2010. Boxplots show mean ± standard deviation. Minimum and maximum values are shown by the upper and lower whiskers. Different letters above each bar indicate a significant difference between mean values (ANOVA, $p < 0.05$).

of infection. Oocysts are easily overlooked, especially in the early stages of infection (after ingestion) when they may occur only in small numbers. Most studies of bark beetle pathogens record only the trophozoite, gamont, and gametocyst stages or do not differentiate these stages at all (Händel *et al.* 2003, Wegensteiner and Weiser 2004, Yaman 2007).

Gregarina typographi is a quite common and non-specific pathogen of bark beetles. About 53% of bark beetle species are hosts to *G. typographi* (Takov *et al.*

2010), and in some cases the prevalence in a population can be high (up to 44%) (Wegensteiner *et al.* 1996, Wegensteiner 2004). The similar level of *G. typographi* infection in the current study area was relatively high. The greater was the density of gallery systems at a given location, the greater was the probability of the pathogen transmission between beetles. Thus, even the recently matured beetles can become infected when they cut through another gallery while feeding (Wegensteiner and Weiser 1996). When their population densities are low, bark beetles only rarely encounter other beetles outside their own galleries, and the spores cannot therefore be ingested with frass or the remnants of dead infected beetles (in the case of infections attacking the fat body) (Wegensteiner and Weiser 1996).

The ratio of infected males and females was similar in the two years, which corresponds to the report by Wegensteiner *et al.* (1996). Both sexes come in contact with contaminated faeces at the same time during breeding, egg-laying and removal of frass from entry holes. These findings show that the risk of infection is similar for both sexes and that the infection spreads more or less evenly among males and females.

Increasing infection level of *G. typographi* is probably the result of mutual transmission of gregarines over a very short time. In both years there was a statistically significant increase in infection levels between the first and last samples. Due to unfavourable weather (low temperatures, snow and rainfall) in 2010 (10 June) a time of the emergence of beetles was later compared to 2009 (26 May). But the time period from the moment of landing of beetles on the logs and excavation of nuptial chambers to their leaving the trap trees (the pupal stage and callow beetles of F1 generation) was similar (about one month) in both years. The duration of the ontogenetic development of gregarines is relatively short, even though the development rate depends on temperature and humidity (MacDougall 1942, Smith *et al.* 2007) or the efficiency of the host's immune system (Corbel 1968). The infection cycle of *Gregarina rigida* (Hall, 1907) Ellis, 1913 requires about 10–11 d from the sporozoites stage to reproductive stage (Allegre 1948). *G. cubensis* Peregrine, 1970 requires more time to complete its development at 27°C (216 h) than at 15°C (144 h) (Smith *et al.* 2007). The temperature of spruce phloem depends on air temperature and solar radiation (Baier *et al.* 2007). During flight activity of bark beetles, the average daily temperature of phloem in shaded areas of the tree stem is balanced around 15°C, while the sunlit parts vary between 15 and 30°C (Netherer *et al.* 2003). We measured a slightly lower bark temperature (7–16°C) due to the high altitude and low temperature of the study site. This means that gregarines' development cycle may be carried out several times during the continued presence of beetles in the breeding. We infer that some infected beetles that were establishing galleries were only in the initial stage of infection and could not transmit the infection to other beetles co-inhabiting the nuptial chamber until sufficient time had passed for *G. typographi* to complete its life cycle. These data explain why the infection level did not increase in the current study unless there were at least 20 d between samplings (see Fig. 4).

The life cycle of species in the genus *Gregarina* Dufour, 1828 is direct, i.e. there is no intermediate host or vector (Clopton and Gold 1996). Gametocysts are shed with the host's frass (Zuk 1987). Upon maturation, gametocysts dehisce to release hundreds of infective oocysts into the environment (Zuk 1987). Faeces along with frass are pushed out from the breeding system by males (Christiansen 2008). During this process, individual oocysts adhere to the walls of the breeding system and are then eaten accidentally when beetles excavate galleries and feed on phloem. Unfortunately, no oocysts were detected in any smear of samples from 50 maternal galleries and displaced frass from 20 entry holes from the sampling date 10 July 2010. Considering the failure to find oocysts in the frass, we assume that the probability of oocysts ingestion was very low in the study population of *I. typographus* and the rise of gregarine infection levels took a longer time than we expected. After ingestion, excystation of gregarines is activated in the gut lumen and the sporozoites leave behind the oocysts (Clopton and Gold 1995). The sporozoite grows into a large trophozoite, the feeding stage of the life cycle. Trophozoites undergo a period of extracellular growth during which they remain attached to the intestinal epithelium by means of an epimerite (Omoto *et al.* 2004, Smith *et al.* 2007, Valigurová *et al.* 2009). Trophozoites as well as gamonts were the most frequently observed stages in the stomodeum and midgut, while gametocysts occurred most frequently in the proctodeum, where they become part of the faeces.

In the Smrk Nature Reserve, where forestry management is limited, beetles are abundant and pathogen transmission is therefore favored. As beetle numbers increase, the level of *G. typographi* infection in *I. typographus* gradually increases (see Holuša *et al.* 2009, Wegensteiner *et al.* 2010). Given what is known about the biology of the pathogen and the beetle, the doubling of the *G. typographi* infection prevalence in 2009 and its more than tripling in 2010 could be a result of disease transmission among beetles in nuptial chambers, i.e. by horizontal transmission during reproduction and egg-laying (May–June).

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REFERENCES

Allegre C. F. (1948) Contributions to the life history of a gregarine parasitic in grasshoppers. *Trans. Am. Microsc. Soc.* **67:** 211–226

Baier P., Pennerstorfer J., Schopf A. (2007) PHENIPS – A comprehensive phenology model of *Ips typographus* (L.) (Col., Scolytinae) as a tool for hazard rating of bark beetle infestation. *Forest Ecol. Manag.* **249:** 171–186

Bjørnson S. (2008) Natural enemies of the convergent lady beetle, *Hippodamia convergens* Guérin-Méneville: Their inadvertent importation and potential significance for augmentative biological control. *Biol. Control* **44:** 305–311

Christiansen E., Bakke A. (1988) The spruce bark beetle of Eurasia. In: Dynamics of forest insect populations, (Ed. A. A. Berryman). Plenum Publishing Corporation, New York and London, 479–503

Christiansen E. (2008) Eurasian spruce bark beetle, *Ips typographus* Linnaeus (Coleoptera: Curculionidae, Scolytinae). In: Encyclopedia of Entomology 2 (Ed. J. L. Capinera). Springer, Dordrecht, 1363–1366

Clopton R. E., Gold R. E. (1995) Effects of pH on excystation of *Gregarina cuneata* and *Gregarina polymorpha* (Eugregarina: Gregarinidae). *J. Eukaryot. Microbiol.* **42:** 540–544

Clopton R. E., Gold R. E. (1996) Host specificity of *Gregarina blattarum* von Siebold, 1839 (Apicomplexa: Eugregarinida) among five species of domiciliary cockroaches. *J. Invertebr. Pathol.* **67:** 219–223

Corbel J. C. (1968) La spécificité parasitaire des Grégarines d'Orthoptères. *Ann. Parasitol. Hum. Comp.* **43:** 25–32

Culek M. (1996) Biogeografické členění České republiky. Enigma, Praha

Forsse E. (1991) Flight propensity and diapause incidence in 5 populations of the bark beetle *Ips typographus* in Scandinavia. *Entomol. Exp. Appl.* **61:** 53–57

Händel U., Wegensteiner R., Weiser J., Žižka Z. (2003) Occurrence of pathogens in associated living bark beetles (Col., Scolytinae) from different spruce stands in Austria. *J. Pest Sci.* **76:** 22–32

Holuša J., Weiser J., Žižka Z. (2009) Pathogens of the spruce bark beetles *Ips typographus* and *Ips duplicatus*. *Cent. Eur. J. Biol.* **4:** 567–573

Kereselidze M., Wegensteiner R., Goginashvili N., Tvaradze M., Pilarska D. (2010) Further studies on the occurrence of natural enemies of *Ips typographus* (Coleoptera: Curculionidae: Scolytinae) in Georgia. *Acta Zool. Bulg.* **62:** 131–139

MacDougall M. M. (1942) A study of temperature effects on gregarines of *Tenebrio molitor* larvae. *J. Parasitol.* **28:** 233–240

Netherer S., Pennerstorfer J., Baier P., Fuhrer E., Schopf A. (2003) Monitoring and risk assessment of the spruce bark beetle, *Ips typographus*. In: Proceedings: Ecology, Survey and Management of Forest Insects, 2002 September 1–5; Krakow, Poland. Gen. Tech. Rep. NE-311 (Eds. M. L. McManus, A. M. Liebhold). Newtown Square, PA: U.S. Dept. of Agriculture, Forest Service, Northeastern Research Station, 75–79

Omoto C. K., Toso M., Tang K., Sibley L. D. (2004) Expressed sequence tag (EST) analysis of Gregarine gametocyst development. *Int. J. Parasitol.* **34:** 1265–1271

Smith A. J., Cook T. J., Lutterschmidt W. I. (2007) Effects of temperature on the development of *Gregarina cubensis* (Apicomplexa: Eugregarinida) parasitizing *Blaberus discoidalis* (Blattaria: Blaberidae). *J. Parasitol.* **93:** 583–588

Takov D., Doychev D., Wegensteiner R., Pilarska D. (2007) Study on the pathogens of bark beetles (Coleoptera, Curculionidae, Scolytinae) from different coniferous stands in Bulgaria. *Acta Zool. Bulg.* **59:** 87–96

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 Zoolog. Bulg.* **62:** 201–209

Valigurová A., Michalková V., Koudela B. (2009) Eugregarine trophozoite detachment from the host epithelium via epimerite retraction: Fiction or fact? *Int. J. Parasitol.* **39:** 1235–1242

Wegensteiner R., Weiser J. (1996) Occurrence of *Chytridiopsis typographi* (Microspora, Chytridiopsida) in *Ips typographus* L. (Col., Scolytinae) field population and in a laboratory stock. *J. Appl. Entomol.* **120:** 595–602

Wegensteiner R., Weiser J., Führer E. (1996) Observations on the occurrence of pathogens in the bark beetle *Ips typographus* L. (Col., Scolytinae). *J. Appl. Entomol.* **120:** 190–204

Wegensteiner R. (2004) Pathogens in bark beetles. In: Bark and wood boring insects in living trees in Europe, A synthesis, (Eds. F. Lieutier, K. R. Day, A. Battisti, J. C. Grégoire, H. F. Evans). Kluwer, Dordrecht, 291–313

Wegensteiner R., Weiser J. (2004) Annual variation of pathogen occurrence and pathogen prevalence in *Ips typographus* L. (Col., Scolytinae) from the BOKU University Forest Demonstration Centre. *J. Pest Sci.* **77:** 221–228

Wegensteiner R., Dedryver C. A., Pierre J. S. (2010) The comparative prevalence and demographic impact of two pathogens in swarming *Ips typographus* adults: a quantitative analysis of long term trapping data. *Agric. Forest. Entomol.* **12:** 49–57

Wermelinger B. (2004) Ecology and management of the spruce bark beetle *Ips typographus* – a review of recent research. *Forest Ecol. Manag.* **202:** 67–82

Yaman M. (2007) *Gregarina typographi* Fuchs, a gregarine pathogen of the six-toothed pine bark beetle, *Ips sexdentatus* (Boerner) (Coleoptera: Curculionidae, Scolytinae) in Turkey. *Turk. J. Zool.* **31:** 359–363

Yaman M. (2008) On the occurrence of a gregarine parasite from *Psylloides cupreus* Koch 1803 (Coleoptera: Chrysomelidae) of Turkey. *North-West. J. Zool.* **4:** 167–172

Zuk M. (1987) The effects of gregarine parasites, body size, and time of day on spermatophore production and sexual selection in field crickets. *Behav. Ecol. Sociobiol.* **21:** 65–72

Received on 21st June, 2011; revised on 21st August, 2011; accepted on 24th August, 2011

4.7 Stanovení vlivu infekčních nákaz na populační růst *Ips typographus* (Coleoptera: Scolytinae) v hospodářských lesích

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Rukopis.

Stanovení vlivu infekčních nákaz na populační růst *Ips typographus* (Coleoptera: Scolytinae) v hospodářských lesích

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Abstrakt

Během osmi generací lýkožrouta smrkového jsme sledovali jeho populační hustoty na 5-10 lapácích na třech studijních lokalitách v České republice. Celkem bylo analyzováno 2591 jedinců *I. typographus* na přítomnost patogenních organismů. Zjištěny byly celkem 4 patogenní organismy: střevní hlístice u 14,8 %, gregarina *Gregarina typographi* 0,3 %, *Chytridiopsis typographi* 9,1 % a larvy endoparazitoidů u 4,9 % jedinců. U střevních hlístic a *Ch. typographi* se potvrdilo, že tyto patogeny neměly vliv na populační růst lýkožrouta smrkového. Oproti tomu mezi koeficientem populačního růstu a procentem brouků napadených endoparazitoidy v populaci byla zjištěna pozitivní korelace. Parazitoidi jsou tak schopni velmi efektivně reagovat na zvýšení populace svého hostitele.

Úvod

Lýkožrout smrkový, *Ips typographus* L. (Coleoptera: Curculionidae: Scolytinae), je jedním z nejvážnějších škůdců smrku ztepilého (*Picea abies* (L.) Karts.) v Eurasii (Annila 1969). Rozmnožuje se na čerstvě odumřelém dříví, ale při velkých populačních hustotách osidluje i zdravé stromy (Weslien et al. 1989). V současnosti se stavy lýkožroutů intenzivně monitorují pomocí klasických metod ochrany lesa. Účinná asanace je však stále založena na odstranění veškerého kůrovcem napadeného i atraktivního materiálu (Wermelinger 2004). Biotické faktory ovlivňující růst nebo pokles populačních hustot nejsou u *I. typographus* stále přesvědčivě vysvětleny, i přesto že se studiu patogenů a predátorů lýkožrouta smrkového zabývala již řada autorů (Kenis et al. 2004; Wegensteiner 2004; Takov et al. 2010). Otázka možnosti využití přirozených nepřátel v biologickém boji je tak nadále otevřená.

U lýkožrouta smrkového je známo deset onemocnění zahrnující prvoky, mikrosporidie a viry (Wegensteiner et al. 1996; Weiser 2002; Wegensteiner 2004; Holuša et al. 2009; Takov et al. 2010). Nejběžnější mikrosporidie je *Chytridiopsis typographi* Weiser (Zygomycota, Microspora), vyskytuje se na většině studovaných lokalit v Evropě (Takov et al. 2010), ovšem s různou hladinou infekce (Händel et al. 2001; Wegensteiner & Weiser 2004; Takov et al. 2007; Holuša et al. 2009; Burjanadze & Goginashvili 2009). Další mikrosporidie jako např. *Nosema typographi* Weiser se objevuji ve velmi nízkých infekčních hladinách řádově v procentech (Händel et al. 2003; Pultar & Weiser 2004). Virus *ItEPV* (Entomopoxviridae) Wegensteiner & Weiser napadá a ničí buňky střevního epitelu lýkožroutů. Destičkovité inkluze s virovými tělísky odcházejí z těla brouků s trusem a infikují další jedince při úživném žíru (Weiser & Wegensteiner 1994; Wegensteiner & Weiser 1995). Hromadinka *Gregarina typographi* Fuchs (Apicomplexa, Eugregarinida) působí pouze slabé poškození střevního epitelu, které navíc částečně regeneruje (Tanada & Kaya 1993). Schizogregariny (Apicomplexa, Neogregarinida) *Menzbiera chalcographi* Weiser a *Mattesia schwenkei* Purrini napadají tukové těleso, které se naplní člunkovitými sporami a nakažení jedinci pravděpodobně umírají pod kůrou stromu (Weiser 2002).

Nezanedbatelnou skupinou patogenů rodu *Ips* jsou hlístice (Nematoda), způsobují ucpání střeva, případně jeho perforaci. Jejich determinace je obtížná, protože se uvnitř těla lýkožroutů objevují často pouze juvenilní jedinci a je nutné je dochovávat (Rühm 1956).

Hojně se mohou vyskytovat také endoparazitoidi lýkožroutů z čeledí Braconidae a Pteromalidae (Hymenoptera). Nejběžnější z nich jsou *Tomicobia seitneri* Ruschka a *Ropalophorus clavicornis* Wesmael (Eck 1990; Faccoli 2001; Wermelinger 2002).

V této studii jsme se zaměřili na hodnocení vlivu patogenů na populační hustoty a růst lýkožrouta smrkového v hospodářských lesích s běžným lesnickým managementem.

Metodika

Během let 2008-2011 byly hodnoceny infekční hladiny patogenů *I. typographus* na třech lokalitách v prostoru Vojenského újezdu Libavá: Potštát (GPS 49°41'21"N, 17°37'11"E); Kozlov (GPS 49°37'58"N, 17°30'16"E) a Staré Odřůvky (GPS 49°43'01"N, 17°36'32"E). Materiál matečných brouků byl shromážděn z 5 až 10 stromů (přilehlé stromy ve vzdálenosti asi 100 m od sebe) na každé lokalitě. Odběry byly prováděny vzhledem k dvěma generacím kůrovců (přezimující a dceřiná) 2x na lokalitách Vojenského újezdu Libavá (červen, srpen). Čas odběru byl určen na základě zahájení nové generace brouků – tyto temíny byly svázány s chodem teplot a srážek během vegetačního období. Na jaře (červen) byli zjišťováni dospělci rodičovské generace (P). V létě proběhla druhá perioda vzorkování (srpen), kde byli nasbíráni brouci dceřiné generace (F1) ze snubních komůrek a matečných chodeb, protože patogeny se snadněji zjišťují u dospělých brouků (Wegensteiner & Weiser 1996a). Pro každou lokalitu a období odběru vzorků bylo shromážděno vždy maximum brouků (minimálně n=20) z každé půlmetrové sekce stromu (celkem čtyři: I. sekce metr od paty stromu, II. sekce – polovina kmene, III. sekce začátek zeleného větvení a IV. sekce zhruba v polovině koruny).

Populační hustota lýkožroutů na jednotlivých sekcích lapáků byla zjišťována při studiu populačních hustot a dalších charakteristik požerků na všech lokalitách během celého výzkumu. Na každé odkorněné sekci byl kalkulován počet rodin (=počet snubních komůrek, stádium vývoje, délka chodeb, počet nakladených vajec a míra parazitace) lýkožrouta smrkového a přepočtena jejich hustota na jednotku plochy podle velikosti studované sekce (délka přibližně 0,5 m a šířka cca polovina obvodu kmene).

Odebraní brouci byli spolu s kouskem navlhčené gázy uloženi v plastových nádobách typu 2-cm^3 Eppendorf do chladu při -5°C. Postupně byli brouci pitváni pomocí chirurgických pinzet. Vzorky byly sledovány pod světelným mikroskopem Arsenal LPE 5013i-T pod zvětšením 100x – 400x. Inspekce se zaměřila především na trávicí soustavu, Malphigické trubice, gonády a tukové těleso, kde jsou patogeny nejčastěji lokalizovány. Z každé pitvy byl veden detailní záznam o stavu a početnosti infekce a vnitřních orgánů. Pozornost je věnována především mikrosporidiím, prvokům, virům a hlísticím.

Pro výpočet korelace mezi infekční hladinou patogenů a nárůstem populační hustoty byl použit koeficient populačního růstu z roku na rok podle vzorce pro výpočet rychlosti růstu:

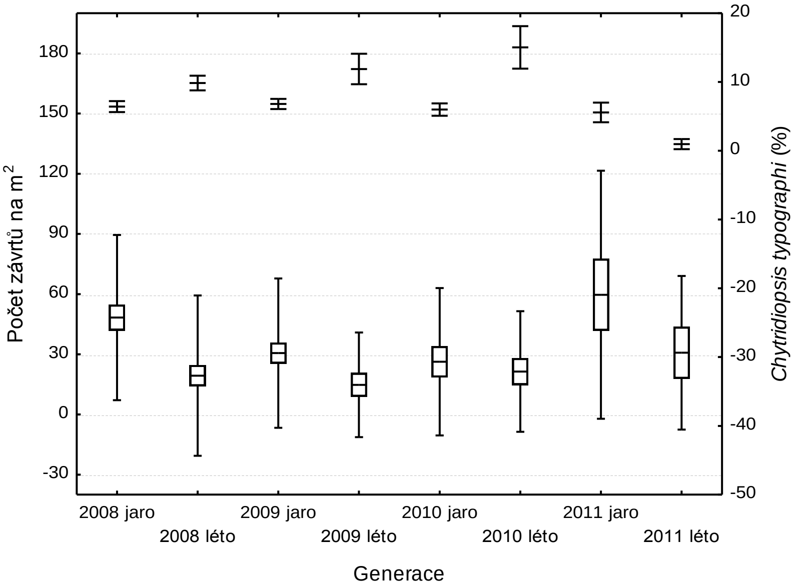
$$R = \log N_t - \log N_{t-1}$$ (Jarošík 2005).

Získaná data jsou převedena do tabulkového softwaru MS Excel 2007 a poté graficky a statisticky vyhodnoceny v programu Statistica 9 (normalita, korelace, ANOVA).

Výsledky

Celkem bylo analyzováno 2591 jedinců *I. typographus* na přítomnost patogenních organismů (Kozlov – 8 generací, n=1310; Potštát – 8 generací, n=780 a Staré Oldřůvky – 4 generace, n=501).

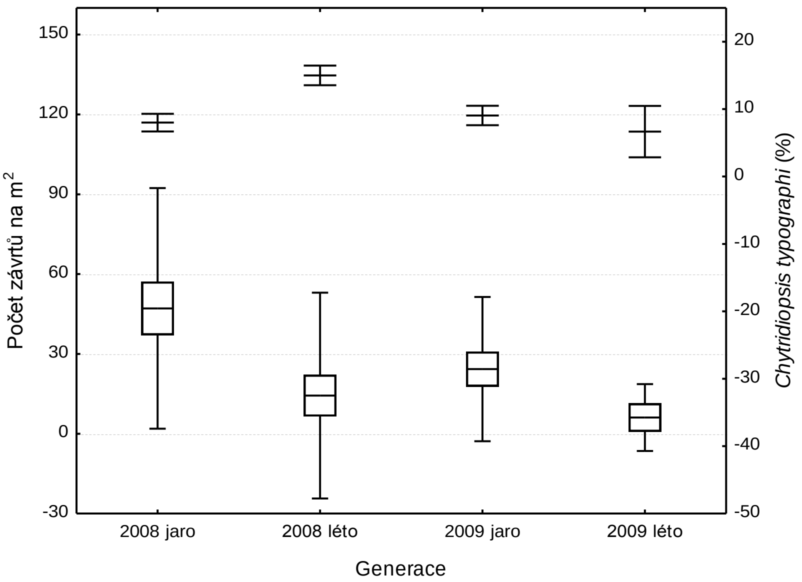
Zjištěny byly celkem 4 patogenní organismy: střevní hlístice u 14,8 ± 11,7 % brouků, *Gregarina typographi* u 0,3 ± 1,0 % brouků, *Chytridiopsis typographi* u 9,1 ± 7,9 % brouků a larvy endoparazitoidů u 4,9 ± 6,3 % brouků.



Graf 1 Infekční hladiny *Chytridiopsis typographi* a populační hustoty *Ips typographus* v letech 2008-2011 na všech lokalitách. Krabice grafu tvoří průměr (čára) a 0,95 interval spolehlivosti, svorky představují směrodatné odchylky základního souboru.

Změny v infekční hladině *Ch. typographi* a populační hustoty lýkožrouta smrkového na všech lokalitách ve všech letech výzkumu ukazuje Graf 1. Infekční hladina *Ch. typographi* byla vždy nižší na jaře (průměrně 6,3 ± 3,0 %) než v létě (průměrně 11,5 ± 6,8 %) s výjimkou roku 2011. Rozdíly mezi generacemi nebyly statisticky signifikantní (Shapiro-Wilk test: W=0,93, p>0,05; ANOVA: F(1, 14)=3,99, p>0,05).

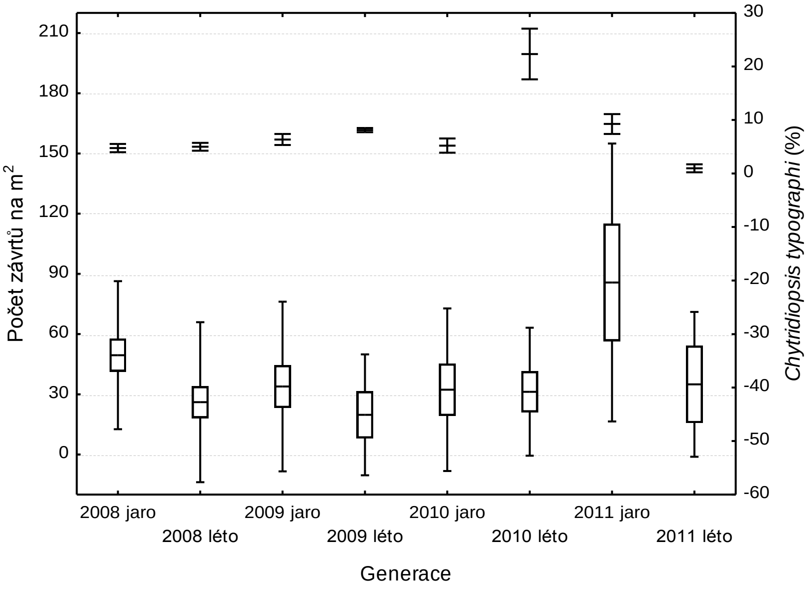
Vývoj infekčních hladin *C. typographi* na jednotlivých lokalitách a populační hustoty *I. typographus* výzkumu shrnují Grafy 2-4.



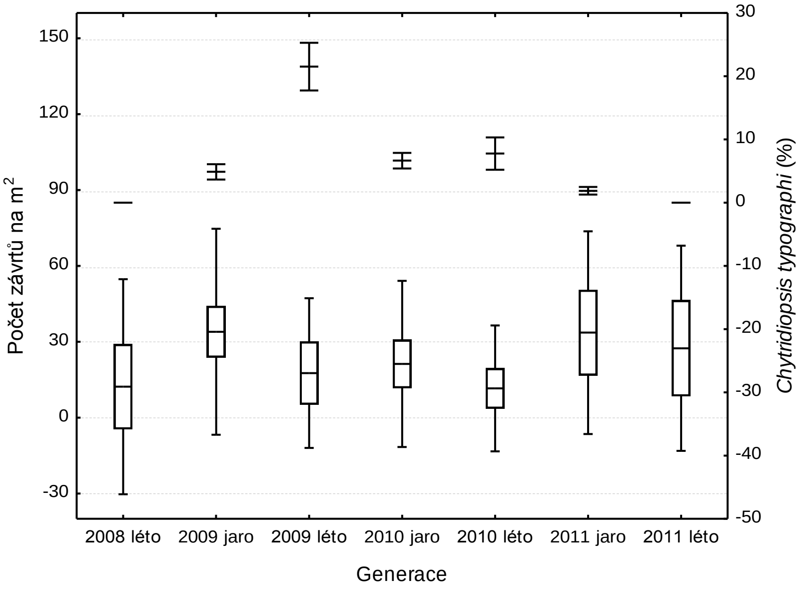
Graf 2 Infekční hladiny *Chytridiopsis typographi* a populační hustoty *Ips typographus* v letech 2008-2009 na lokalitě Staré Oldřůvky. Krabice grafu tvoří průměr (čára) a 0,95 interval spolehlivosti, svorky představují směrodatné odchylky základního souboru.

Byly vypočteny hodnoty korelačních koeficientů mezi koeficientem populačního růstu lýkožrouta smrkového a infekční hladinou 3 patogenních organismů na 2 lokalitách (Potštát a Kozlov). Nebyl zjištěn vliv střevních hlístic (lokalita Potštát: y=9,34+1,15*x; r=0,05; p>0,05; r^2=0,003; lokalita Kozlov: y=13,18-8,42*x; r=-0,54; p>0,05; r^2=0,30), ani mikrosporidie *Chytridiopsis typographi* (lokalita Potštát: y=6,77-2,15*x; r=-0,14; p>0,05; r^2=0,02; lokalita Kozlov: y=9,46+9,72*x; r=0,62; p>0,05; r^2=0,39), ani endoparazitoidů (lokalita Potštát: y=5,70+13,92*x; r=0,75; p>0,05; r^2=0,57; lokalita Kozlov: y=2,63+4,32*x; r=0,50; p>0,05; r^2=0,25) na populační růst lýkožrouta smrkového.

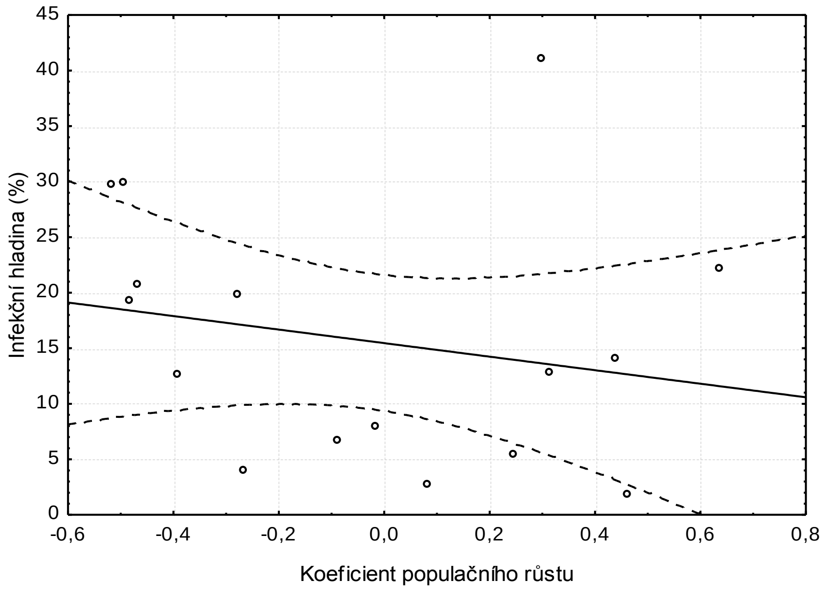
Analýza byla znovu provedena po sloučení dat ze všech tří lokalit (Potštát, Kozlov, Staré Oldřůvky). U střevních hlístic a *Ch. typographi* se potvrdilo, že tyto patogeny neměly vliv na populační růst lýkožrouta smrkového (Graf 5 a 6). Oproti tomu mezi koeficientem populačního růstu a infekční hladinou endoparazitoidů v populaci byla zjištěna pozitivní korelace (Graf 7). Tedy s populačním růstem lýkožrouta smrkového roste i počet jedinců napadených endoparazitoidy.



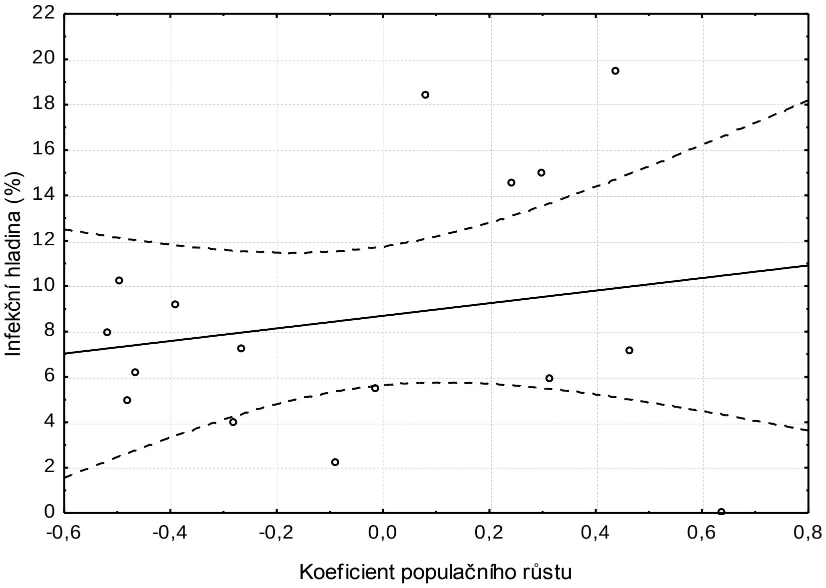
Graf 3 Infekční hladiny *Chytridiopsis typographi* a populační hustoty *Ips typographus* v letech 2008-2011 na lokalitě Kozlov. Krabice grafu tvoří průměr (čára) a 0,95 interval spolehlivosti, svorky představují směrodatné odchylky základního souboru.



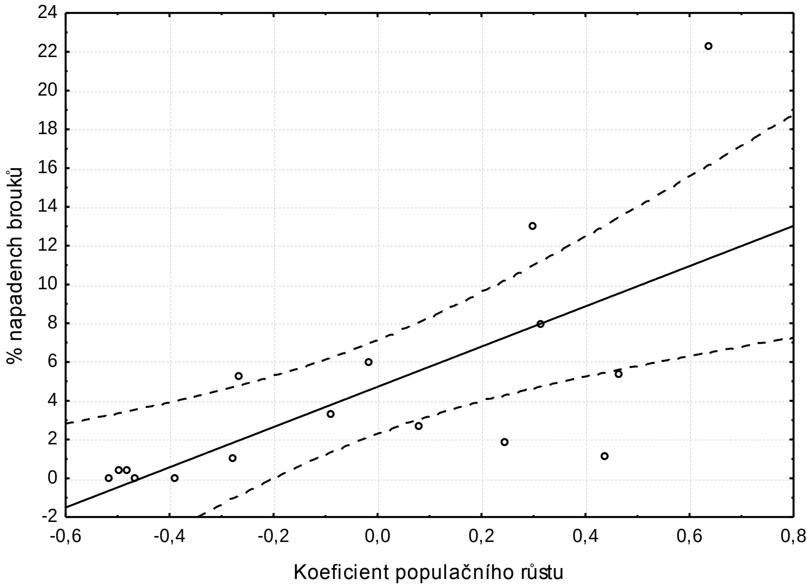
Graf 4 Infekční hladiny *Chytridiopsis typographi* a populační hustoty *Ips typographus* v letech 2008-2011 na lokalitě Potštát. Krabice grafu tvoří průměr (čára) a 0,95 interval spolehlivosti, svorky představují směrodatné odchylky základního souboru.



Graf 5 Závislost průměrných infekčních hladin střevních hlístic na koeficientu populačního růstu *Ips typographus* (pásy…0,95 interval spolehlivosti): y =15,46-6,09*x; r=-0,21; p>0,05; r2=0,05).



Graf 6 Závislost průměrných infekčních hladin *Chytridiopsis typographi* na koeficientu populačního růstu *Ips typographus* (pásy…0,95 interval spolehlivosti): y=8,69+2,78*x; r=0,19; p>0,05; r2=0,04).



Graf 7 Závislost výskytu larev endoparazitoidů na koeficientu populačního růstu *Ips typographus* (pásy…0,95 interval spolehlivosti): y=4,72+10,38*x; r=0,68; p<0,01; r2=0,47).

Diskuse

Na lokalitách s omezeným lesnickým managementem s ohnisky přemnožení kůrovců dochází k intenzivnímu kontaktu brouků během zralostního žíru se sporami a cystami patogenů (Holuša et al. 2009). Infekční hladiny nemocí pak ještě více narůstají mezi brouky ve snubních komůrkách a během tvorby požerku (Lukášová & Holuša 2011). Při nízkých populačních denzitách ještě umocněných včasnými obrannými opatřeními proti kůrovcům, jako byly zjištěny v našem výzkumu (počet rodin jen zřídka překročil 1 na dm^2), se lýkožrouti setkávají s jedinci mimo svůj požerek jen velmi zřídka a k předávání mimo přímý kontakt s nakaženými matečnými brouky dochází většinou zcela náhodně (Wegensteiner & Weiser 1996a). To v naší studii vysvětluje absenci patogenů napadajících tukové těleso (např. mikrosporidie *Nosema typographi* nebo neogregariny *Menzbiera chalcographi* a *Mattesia schwenkei*), jejichž spory se uvolňují až po odumření hostitele a k přenosu dochází v době, kdy už většina brouků rodinné požerky opustila (Weiser 2002).

Protože infekční hladina eugregariny *Gregarina typographi* byla velmi nízká, nemůžeme tak předpokládat žádný vliv na populační růst na studovaných lokalitách. Obecně se také předpokládá, že se nejedná o příliš virulentní patogen (Yaman 2007; Wegensteiner et al. 2010). Na jednu stranu se považuje za komenzála (Weiser & Briggs 1971), na druhou stranu však údajně ničí buňky střevního epitelu ovšem bez letálních účinků (Zuk 1987; Thomas et al. 1999). Na populace lýkožrouta smrkového nebyl zatím touto gregarinou zjištěn žádný demografický efekt (Wegensteiner et al. 2010).

Stejně tak většina hlístic asociovaných s lýkožroutem smrkovým brouky negativně neovlivňuje, ačkoli existují některé parazitické druhy (Rühm 1956). Podle některých studií zabíjí parazitické hlístice své hostitele a redukují jejich životnost a také plodnost (Fuchs 1915; Lieutier 1980; Kaya 1984), např. zmenšením oocytů napadených samic lýkožroutů (Thong & Webster 1975). Průměrná nákaza na studovaných lokalitách varírovala kolem 15 %, což je nižší hladina než v dalších výzkumech, kde se pohybuje kolem 50% (Wegensteiner & Weiser 1996b; Burjanadze & Goginashvili 2009; Kereselidze et al. 2010). Ze získaných výsledků a neprůkazné korelace můžeme usoudit, že hlístice populační růst neovlivňují nebo mají jen minimální dopad.

Průměrné infekční hladiny mikrosporidie *Ch. typographi* varírovaly od 0 do 20,4 %. Toto chronické onemocnění je lokalizované v epitelu středního střeva (Wegensteiner 2004). Infekční hladiny tohoto patogenu nekorelovaly s populačním růstem, což znamená, že populace kůrovce dlouhodobě neovlivňují. Zřejmě nezpůsobují vysokou mortalitu nebo zapřičiňují úhyn matečných brouků až po úspěšném rozmnožování a vykladení vajíček. Můžeme předpokládat, že pouze při silné infekci může dojít pouze k ucpání střeva, které navíc může nastat až po období rozmnožování a nedochází k snížené plodnosti. V jarních odběrech přezimující generace byly infekční hladiny tohoto patogenu vždy nižší než v letní generaci. Tento patogen se totiž šíří poměrně rychle z rodičovské na dceřinou generaci (Wegensteiner et al. 1996) transovariálním přenosem z matky přes vajíčka na potomstvo (Weiser et al. 2000).

Tomicobia seitneri jako jeden z nejčastějších endoparazitoidů reaguje na agregační feromon vylučovaný *I. typographus* (Faccoli 2000). Vzhledem k obtížně determinaci larev byly druhově zařazeny pouze larvy 1. instaru. Námi zjištěná parazitace pohybující se kolem 5 % všech endoparazitoidů přítomných v broucích (všechna stádia, tzn. vajíčka, larvy, kukly) byla zcela v rozsahu již dříve zjištěných dat (Faccoli 2000; 2001; Wermelinger 2002; Feicht 2004; Faccoli & Bernardinelli 2011). Úmrtnost kůrovců způsobená parazitoidy (zahrnující endoparazitoidy dospělců i ektoparazitoidy larev) se pohybuje nad 30% (Weslien 1992; Eck 1990). Parazitoidi se vyznačují vysokou hostitelskou specifitou, jsou citliví na změny v prostředí a preferují tenčí kůru na kmeni (Jonsell et al. 1999; Wermelinger 2002; Ulyshen &

Pucci 2010). Své hostitele vyhledávají pomocí semiochemikálií vypouštěnými kůrovci a volatilních látek uvolňovanými napadeným stromem (Pettersson 2001). Celková míra parazitace a tedy i mortalita se často pohybuje kolem 10 % (Feicht 2004), za určitých okolností ale dosahuje 20-50% a více (Faccoli 2000).

Pozitivní korelace mezi populační hustotou kůrovce a procentem napadení endoparazitoidy v naší studii souvisí zřejmě se schopností parazitoidů rychle reagovat a přizpůsobit se změnám ve velikosti populace kůrovců (Feicht 2004). Při populačním růstu lýkožroutů se tedy i zvýšil výskyt endoparazitických blanokřídlých v matečných broucích.

Použitá literatura

Annila E. 1969: Influence of temperature opon the development and voltinism of *Ips typographus* L. (Coleoptera, Scolytidae). Annales Zoologici Fennici, 6: 161-208.

Burjanadze M. & Goginashvili N. 2009: Occurrence of pathogens and nematodes in the spruce bark beetles, *Ips typographus* (Col., Scolytidae) in Borjomi Gorge. Bulletin of the Georgian National Academy of Sciences, 3: 145-150.

Eck R. 1990: Die parasitischen Hymenopteran des *Ips typographus* in der Phase der Progradation. Entomologische Abhandlungen, 53: 152-178.

Faccoli M. 2000: Notes on the biology and ecology of *Tomicobia seitneri* (Ruschka) (Hymenoptera: Pteromalidae), a parasitoid of *Ips typographus* (L.) (Coleoptera: Scolytidae). Frustula Entomologica, 23: 47-55.

Faccoli M. 2001: *Tomicobia seitneri* (Ruschka), *Ropalophorus clavicornis* (Wesmael) and *Coeloides bostrychorum* Giraud: three hymenopterous parasitoids of *Ips typographus* (L.) (Col., Scolytidae) new to Italy. Bollettino della Societa Entomologica Italiana, 133: 237-246.

Faccoli M. & Bernardinelli I. 2011: Breeding performance of the second generation in some bivoltine populations of *Ips typographus* (Coleoptera Curculionidae) in the south-eastern Alps. Journal of Pest Science, 84: 15-23.

Feicht E. 2004: Parasitoids of *Ips typographus* (Col., Scolytidae), their frequency and composition in uncontrolled and controlled infested spruce forest in Bavaria. Journal of Pest Science, 77: 165-172.

Fuchs A. G. 1915: Die Naturgeschichte der Nematoden und einiger anderer Parasiten. 1. Des *Ips typographus* L. 2. Des *Hylobius abietis* L.. Zoologishe Jahrbücher, Abteilung für Systematik, Öekologie und Geographie der Tiere, 38: 109-222.

Händel U., Kenis M. & Wegensteiner R. 2001: Untersuchungen zum Vorkommen von Pathogenen und Parasiten in Populationen überwinternder Fichtenborkenkäfer (Col., Scolytidae). Mitteilungen der Deutschen Gesellschaft für Algemeine und Angewandte Entomologie, 13: 423-428.

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.

Holuša J., Weiser J. & Žižka Z. 2009: Pathogens of the spruce bark beetles *Ips typographus* and *Ips duplicatus*. Central European Journal of Biology, 4: 567-573.

Jarošík V. 2005: Růst a regulace populací. Vyd. 1. Academia, Praha. 170 pp.

Jonsell M., Nordlander G. & Jonsson M. 1999: Colonization patterns of insects breeding in wood-decaying fungi. Journal of Insect Conservation, 3: 145-161.

Kaya H. K. 1984: Nematode parasites of bark beetles. pp. 727-754. In: Nickle W.R. (ed.) Plant and Insect Nematodes. Marcel Dekker, Inc., New York and Basel.

Kenis B., Wermelinger & Grégoire J.C. 2004: Natural enemies of bark beetles. 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. Kluwer, Dordrecht.

Kereselidze M., Wegensteiner R., Goginashvili N., Tvaradze M. & Pilarska D. 2010: Further studies on the occurrence of natural enemies of *Ips typographus* (Coleoptera: Curculionidae: Scolytinae) in Georgia. Acta Zoologica Bulgarica, 62: 131-139.

Lieutier F. 1980: Le parasitisme d'*Ips sexdentatus* (Boern) (Coleoptera: Scolytidae) par les nematodes du genre *Parasitaphelenchus* Fuchs. Relations aves le parasitisme par *Contortylenchus diplogaster* (v. Lins.). Rewue de Nematologie, 3: 271-281.

Lukášová K. & Holuša J. 2011: *Gregarina typographi* (Eugregarinorida: Gregarinidae) in the bark beetle *Ips typographus* (Coleoptera: Curculionidae): changes in infection level in the breeding system. Acta Protozoologica, 50: 311-318.

Pettersson E. M. 2001: Volatiles from potential host of *Rhopalicus tutela* a bark beetle parasitoid. Journal of Chemical Ecology, 27: 2219-2217.

Pultar O. & Weiser J. 2004: Výsledky posledních patologických studií dominantních kůrovců v NP Šumava a jejich využití. In: Sborník referátů 28. setkání lesníků tří generací na téma „Nebezpečí kůrovce v roce 2004“ 19. 3. 2004 (Praha-Novotného lávka), Česká lesnická společnost, VÚLHM Jíloviště-Strnady, Lesnická práce.

Rühm W. 1956: Die Nematoden der Ipiden. Parasitologische Schriftenreihe, 6: 1-437.

Takov D., Doychev D., Wegensteiner R. & Pilarska D. 2007: Study on the pathogens of bark beetles (Coleoptera, Scolytidae) from different coniferous stands in Bulgaria. Acta Zoologica Bulgarica, 59: 87-96.

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.

Tanada Y. & Kaya H. K. 1993: Protozoan infection: Apicomplexa, Microspora. pp. 414-458. In: Tanada Y. & Kaya H. K. (eds.) Insect Pathology. Academic Press, San Diego.

Thomas F., Oget E., Gente P., Desmots D. & Renaud F. 1999: Assortative pairing with respect to parasite load in the beetle *Timarcha maritima* (Chrysomelidae). Journal of Evolutionary Biology, 12: 385-390.

Thong C. H. S. & Webster J. M. 1975: Effects of bark beetle nematode, *Contortylenchus reversus*, on gallery construction, fecundity, and egg viability of douglas-fir beetle, *Dendroctonus pseudotsugae* (Coleoptera-Scolytidae). Journal of Invertebrate Pathology, 26: 235-238.

Ulyshen M. D. & Pucci T. M. 2010: The importace of forest type, tree species and wood posture to saproxylic wasp (Hymenoptera) communities in the southeastern United States. Journal of Insect Conservation, 10: 5-10.

Wegensteiner R. 2004: Pathogens in bark beetles. pp. 291-313. In: Lieutier F., Day K., Battisti A., Gregoire J.-C. & Evans H. (eds.) European Bark and Wood Boring Insects in Living Trees, a Synthesis. Kluwer Academic Publishers, Dordrecht.

Wegensteiner R., Dedryver C.-A. & Pierre J.-S. 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. 1995: A new entomopoxvirus in the bark beetle *Ips typographus* (Coleoptera, Scolytidae). Journal of Invertebrate Pathology, 65: 203-205.

Wegensteiner R. & Weiser J. 1996a: Occurrence of *Chytridiopsis typographi* (Microspora, Chytridiopsida) in *Ips typographus* L. (Col., Scolytidae) field population and in a laboratory stock. Journal of Applied Entomology, 120: 595-602.

Wegensteiner R. & Weiser J. 1996b: Untersuchungen zum Auftreten von Pathogenen bei *Ips typographus* L. (Coleoptera, Scolytidae) aus einem Naturschutzgebiet im Schwarzwald (Baden-Württemberg). Anzeiger für Schädlingskunde, Pflanzenschutz, Umweltschutz, 69: 162-167.

Wegensteiner R. & Weiser J. 2004: Annual variation of pathogen occurrence and pathogen prevalence in *Ips typographus* L. (Col, Scolytidae) from the BOKU University Forest Demonstration Centre. Journal of Pest Science, 77: 221-228.

Wegensteiner R., Weiser J. & Führer E. 1996: Observations on the occurrence of pathogens in the bark beetle *Ips typographus* L. (Col., Scolytidae). Journal Applied Entomology, 120: 190-204.

Weiser J. 2002: Patogenní organismy. In Skuhravý V. : Lýkožrout smrkový (*Ips typographus* L.) a jeho kalamity. Der Buchdrucker und seine Kalamitäten. Agrospoj, Praha, pp. 97-100.

Weiser J. & Briggs J. D. 1971: Identification of pathogens. pp. 13-66. In: Burges H. D. & Hussey N. W. (eds.) Microbial control of insects and mites. Academic Press, London.

Weiser J., Pultar O. & Žižka Z. 2000: Biological protection of forest against bark beetle outbreaks with poxvirus and other pathogens. IUAPPA, Section B, 12: 168-172.

Weiser J. & Wegensteiner R. 1994: A new type, EPV-Ab of the entomopox virus in the bark beetle *Ips typographus* in Czechoslovakia. Zeitschrift für Angewandte Entomologie, 80: 425-434.

Wermelinger B. 2002: Development and distribution of predators and parasitoids during two consecutive years of an *Ips typographus* (Col., Scolytidae) infestation. Journal of Applied Entomology, 126: 521-527.

Wermelinger B. 2004: Ecology and management of the spruce bark beetle *Ips typographus*—a review of recent research. Forest Ecology and Management, 202: 67-82.

Weslien J. 1992: The arthropod complex associated with *Ips typographus* (L.) (Coleoptera, Scolytidae): species composition, phenology, and impact on bark beetle productivity. Entomologica Fennica, 3: 205-213.

Weslien J., Annila E., Bakke A., Bejer B., Eidmann H. H., Narvestad K., Nikula A. & Ravn H. P. 1989: Estimating risk for spruce bark beetle (*Ips typographus* (L.)) damage using pheromone-baited traps and trees. Scandinavian Journal of Forest Research, 4: 87-98.

Yaman M. 2007: *Gregarina typographi* Fuchs, a gregarine pathogen of the six-toothed pine bark beetle, *Ips sexdentatus* (Boerner) (Coleoptera: Curculionidae, Scolytinae) in Turkey. Turkish Journal of Zoology, 31: 359-363.

Zuk M. 1987: The effects of gregarine parasites, body size, and time of day on spermatophore production and sexual selection in field crickets. Behavioral Ecology and Sociobiology, 21: 65-72.

5 Závěry a doporučení pro využití poznatků v praxi

Řešení tématu přispělo k hlubšímu poznání účinků patogenů na populace lýkožroutů rodu *Ips*. Byl vytvořen přehled a rešerše všech poznatků o nemocech kůrovců rodu *Ips*. Byl analyzován výskyt a abundance *Ips amitinus* v různých nadmořských výškách. Patogeny lýkožrouta menšího - *I. amitinus* byly srovnány s lýkožroutem smrkovým *I. typograhus,* zároveň byla u *I. amitinus* poprvé nalezena neogregarina *Mattesia schwenkei* z materiálu získaného v roce 2009 na Šumavě.

U druhu *I. cembrae* bylo poprvé definováno dosud nepopsané druhové složení patogenů a infekční hladiny nákaz ve střední Evropě (Česká republika, Polsko, Rakousko, Chorvatsko). Celkem byl potvrzen výskyt 4 běžných patogenů. Na všech lokalitách byla zjištěna běžná mikrosporidie *Chytridiopsis typographi*, dále pak na dvou lokalitách *Gregarina typographi* a *Nosema* sp. *Mattesia schwenkei* byla nalezena pouze u *I. cembrae* v Chorvatsku. Specifický patogen nebyl potvrzen (analyzováno více než 3 000 jedinců *I. cembrae*). Mimo druhového složení patogenů byla ucelena bionomie tohoto druhu formou odborného článku (momentálně v recenzním řízení v časopise Šumarski list).

V rámci dizertační práce byl nepřímo prokázán horizontální přenos patogenů (gregariny) ve snubních komůrkách v rámci jedné generace brouků, kdy se infekční hladina nemoci zvedla více než 3x. Analýzou deseti generací lýkožrouta smrkového a jeho patogenů byl zjišťován vliv nákaz na populační hustotu a růst. Korelace nebyla prokázána u hlístic ani mikrosporidie *Chytridiopsis typographi*, tyto patogeny nemají dostatečný vliv na populace kůrovce, aby mohly být použitelné v biologickém boji s tímto škůdcem.

Vzhledem k předběžným výsledkům dizertační práce je patrné, že patogeny lýkožroutů nejsou schopny dostatečně regulovat populace těchto druhů. S přihlédnutím k velmi obtížné aplikaci v praxi (kultivace patogenů, kryptický způsob života kůrovců, v případě nízkých populačních hustot také téměř nulový přenos patogenů) lze považovat potenciální využití výše zmíněných patogenů jako jednoho z obranných opatření vedoucích ke snížení populačních hustot, vitality a fertility kůrovců za značně nedostačující.

Původní výsledky byly a budou publikovány ve vědeckých časopisech (databáze Scopus a Web of Science) a prezentovány na odborných českých (Zoologické dny 2010-2012) a zahraničních konferencích (3. Meeting of Forest Protection Experts and Forest Phytosanitary Experts, Vídeň, Rakousko, 2009; IXth European Congress of Entomology, Budapešť, Maďarsko, 2010; IUFRO WP.7.03.05 - Ecology and Management of Bark and Wood Boring Insects: Novel risks with bark and wood boring insects in broadleaved and conifer forests, Sopron, Maďarsko, 2011; Applied Forestry Research in the 21st Century. International conference held on the occasion of the 90th anniversary of the Forestry and Game Management Research Institute. Praha-Průhonice, 2011).

6 Použitá literatura

Allegre C. F. 1948: Contributions to the life history of a gregarine parasitic in grasshoppers. Transactions of the American Microscopical Society, 67: 211-226.

Annila E. 1969: Influence of temperature opon the development and voltinism of *Ips typographus* L. (Coleoptera, Scolytidae). Annales Zoologici Fennici, 6: 161-208.

Annila E. & Nuorteva M. 1977: Dates of attack and emergence of *Ips amitinus* Eichh. (Col., Scolytidae) in Finland. Annales Entomologici Fennici, 43: 28-30.

Baier P., Pennerstorfer J. & Schopf A. 2007: PHENIPS – A comprehensive phenology model of *Ips typographus* (L.) (Col., Scolytinae) as a tool for hazard rating of bark beetle infestation. Forest Ecology Management, 249: 171-186.

Bakke A. 1968: Ecological studies on bark beetles (Coleoptera: Scolytidae) associated with Scots pine (*Pinus sylvestris* L.) in Norway with particular reference to the influence of temperature. Meddelelser fra Norsk Institutt for Skogforskning, 21: 443-602.

Barta J. R. & Thompson R. C. 2006: What is *Cryptosporidium*? Reappraising its biology and phylogenetic affinities. Trends in Parasitology, 22: 463-468.

Bathon H. 1996: Impact of entomopathogenic nematodes on non-target hosts. Biocontrol Science and Technology, 6: 421-434.

Besceli O. & Ekici M. 1969: Control and biology of *Ips sexdentatus* in the *Picea orientalis* region. Ormancilik Arastirma Enstitusu, Teknik Bulten, 32 pp.

Bevan D. 1987: Forest insects. A guide to insects feeding on trees in britain. Forestry Commission, Handbook 1. HMSO, London, 153 pp.

Bidochka M. J., Menzies F. V. & Kamp A. M. 2002: Genetic groups of the insect-pathogenic fungus *Beauveria bassiana* are associated with habitat and thermal growth preferences. Archives of Microbiology, 178: 531-537.

Biermann G. & Thalenhorst W. 1977: Zur Kenntnis des kleinen Buchdruckers, *Ips amitinus* (Eichh.) (Col., Scolytidae). Anzeiger für Schädlingskunde, Pflanzenschutz und Umweltschutz, 50: 20-23.

Bjørnson S. 2008: Natural enemies of the convergent lady beetle, *Hippodamia convergens* Guérin-Méneville: Their inadvertent importation and potential significance for augmentative biological control. Biological Control, 44: 305-311.

Bohne W., Ferguson D. J., Kohler K. & Gross U. 2000: Developmental expression of a tandemly repeated, glycine- and serine-rich spore wall protein in the microsporidian pathogen *Encephalitozoon cuniculi*. Infection and Immunity, 68: 2268-2275.

Brosson D., Kuhn L., Prensier G., Vivares C. P. & Texier C. 2005: The putative chitin deacetylase of *Encephalitozoon cuniculi*: a surface protein implicated in microsporidian spore-wall formation. Fems Microbiology Letters, 247: 81-90.

Burjanadze M. & Goginashvili N. 2009: Occurrence of pathogens and nematodes in the spruce bark beetles, *Ips typographus* (Col., Scolytidae) in Borjomi Gorge. Bulletin of the Georgian National Academy of Sciences, 3: 145-149.

CABI/EPPO 1997: Quarantine Pests for Europe. 2nd ed. In: Smith I. M., McNamara D. G., Scott P. R. & Holderness M. (eds.) CAB International. Wallingford, 1425 pp.

Cali A. & Takvorian P. 1999: Developmental morphology and life cycles of the microsporidia. In: Wittner M. & Weiss L. (eds.) The Microsporidia and Microsporidiosis. American Society of Microbiology, Washington DC, pp. 85-128.

Ceryngier P. & Hodek I. 1996: Enemies of Coccinellidae. In: Hodek I. & Honěk A. (eds.) Ecology of Coccinellidae. Kluwer Academic Publishers, Boston, pp. 319-350.

Christiansen E. 2008: Eurasian spruce bark beetle, *Ips typographus* Linnaeus (Coleoptera: Curculionidae, Scolytinae). In: Capinera J. L. (ed.) Encyclopedia of Entomology 2. Springer, Dordrecht, pp. 1363-1366.

Christiansen E. & Bakke A. 1988: The spruce bark beetle of Eurasia. In: Berryman A. A. (ed.) Dynamics of Forest Insect Populations. Plenum Press, New York, pp. 479-503.

Clopton R. E. 2000: Phylum Apicomplexa Levine, 1970: order Eugregarinorida Léger, 1900. In: Lee J. J., Leedale G., Patterson D. & Bradbury P. C. (eds.) Illustrated guide to the protozoa, 2nd Ed. Society of protozoologists, Lawrence, pp. 205-288.

Clopton R. E. & Gold R. E. 1995: Effects of pH on excystation of *Gregarina cuneata* and *Gregarina polymorpha* (Eugregarina: Gregarinidae). Journal of Eukaryotic Microbiology, 42: 540-544.

Clopton R. E. & Gold R. E. 1996: Host specificity of *Gregarina blattarum* von Siebold, 1839 (Apicomplexa: Eugregarinida) among five species of domiciliary cockroaches. Journal of Invertebrate Pathology, 67: 219-223.

Coeln M., Yanzhang M. & Führer E. 1996: Entwicklung von Fichtenborkenkäfern in Abhängigkeit von thermischen Bedingungen verschiedener montaner Waldstufen (Coleoptera: Scolytidae). Entomologia Generalis, 21: 37-54.

Coulson J. R., Vail P. V., Dix M. E., Nordlund D. A. & Kaufmann W. C. (eds.) 2000: 110 years of biological control research and development in the United States Dept. of Agriculture (1883-1993). U.S. Department of Agriculture. Agricultural Research Service, Washington DC, 645 pp.

Corbel J. C. 1968: La spécificité parasitaire des Grégarines d'Orthoptères. Annales de Parasitologie Humaine et Comparee, 43: 25-32

Corradi N. & Keeling P. J. 2009: Microsporidia: a journey through radical taxonomical revisions. Fungal Biology Reviews, 23: 1-8.

Croll N. A. 1970: The behavior of nematodes. St. Martin's Press, New York, 117 pp.

Culek M. 1996: Biogeografické členění České republiky. Enigma, Praha, 244 pp.

Čapek M. 1994: Biologický boj. In: Lesnický naučný slovník. MZe, Praha, pp. 88-89.

Delbac F. & Polonais V. 2008: The microsporidian polar tube and its role in invasion. Subcellular Biochemistry, 47: 208-220.

Dominik J. 2003: Research on damage of exotic coniferous species caused by indigenous insects – supplementary results. Sylwan 10: 25-28.

Draganova S., Takov D. & Doychev D. 2007: Bioassays with isolates of *Beauveria bassiana* (Bals.) Vuill. and *Paecilomyces farinosus* (Holm.) Brown, Smith against *Ips sexdentatus* Boerner and *Ips acuminatus* Gyll. (Coleoptera: Scolytidae). Plant Science, 44: 24-28.

Duncan L. W. & McCoy C. W. 1996: Vertical distribution in soil, persistence, and efficacy against citrus root weevil (Coleoptera: Curculionidae) of two species of entomogenous nematodes (Rhabditida: Steinernematidae: Heterorhabditidae). Environmental Entomology, 25: 174-178.

Eck R. 1990: Die parasitischen Hymenopteran des *Ips typographus* in der Phase der Progradation. Entomologische Abhandlungen, 53: 152-178.

Elsner G. 1997: Relationships between cutting time in winter and breeding success of *Ips cembrae* in larch timber. Mitteilungen der Deutschen Gesellschaft für allgemeine und angewandte Entomologie, 11: 653-657.

EPPO/CABI 1997: Qurantine Pests for Europe. In: Data sheets on quarantine pests for the European Union and for the European and Mediterranean Plant Protection Organization.- Second Edition. In: Smith I. M., McNamara D. G., Scott P. R. & Holderness M. (eds.) CAB International. Wallingford, 1425 pp.

Faccoli M. 2000: Notes on the biology and ecology of *Tomicobia seitneri* (Ruschka) (Hymenoptera: Pteromalidae), a parasitoid of *Ips typographus* (L.) (Coleoptera: Scolytidae). Frustula Entomologica, 23: 47-55.

Faccoli M. 2001: *Tomicobia seitneri* (Ruschka), *Ropalophorus clavicornis* (Wesmael) and *Coeloides bostrychorum* Giraud: three hymenopterous parasitoids of *Ips typographus* (L.) (Col., Scolytidae) new to Italy. Bollettino della Societa Entomologica Italiana, 133: 237-246.

Faccoli M. & Bernardinelli I. 2011: Breeding performance of the second generation in some bivoltine populations of *Ips typographus* (Coleoptera Curculionidae) in the south-eastern Alps. Journal of Pest Science, 84: 15-23.

Feicht E. 2004: Parasitoids of *Ips typographus* (Col., Scolytidae), their frequency and composition in uncontrolled and controlled infested spruce forest in Bavaria. Journal of Pest Science, 77: 165-172.

Fielding N. J. & Evans H. F. 1997: Biological control of *Dendroctonus micans* (Scolytidae) in Great Britain. Biocontrol, 18: 51-60.

Fielding N. J., O’Keefe T. & King C. J. 1991: Dispersal and host-finding capability of the predatory beetle *Rhizophagus grandis* Gyll. (Col: Rhizophagidae). Journal of Applied Entomology, 112: 89-98.

Forsse E. 1987: Flight duration in *Ips typographus* L.: insensitivity to nematode infection. Journal of Applied Entomology, 104: 326-328.

Forsse E. 1991: Flight propensity and diapause incidence in 5 populations of the bark beetle *Ips typographus* in Scandinavia. Entomologia Experimentalis et Applicata, 61: 53-57.

Forst S., Dowds B., Boemare N. & Stackebrandt E. 1997: *Xenorhabdus* and *Photorhabdus* spp.: bugs that kill bugs. Annual Review of Microbiology, 51: 47-72.

Francke W., Sauerwein P., Vité J. P. & Klimetzek D. 1980: The pheromone bouquet of *Ips amitinus*. Naturwissenschaften, 67: 147.

Francke W. & Vité J. P. 1983: Oxygenated terpenes in pheromone systems of bark beetles. Zeitschrift für Angewandte Entomologie, 96: 146-156.

Franzen C. 2004: Microsporidia: how can they invade other cells? Trends in Parasitology, 20: 275-279.

Franzen C. 2005: How do microsporidia invade cells? Folia Parasitologica, 52: 36-40.

Fuchs A. G. 1915: Die Naturgeschichte der Nematoden und einiger anderer Parasiten. 1. Des *Ips typographus* L. 2. Des *Hylobius abietis* L.. Zoologishe Jahrbücher, Abteilung für Systematik, Öekologie und Geographie der Tiere, 38: 109-222.

Gaugler R. 2002: Entomopathogenic Nematology. CABI Publishing, Wallingford, 372 pp.

Gaugler R. & Kaya H. K. 1990: Entomopathogenic Nematodes in Biological Control. CRC Press, Boca Raton, 365 pp.

Glowacka B. 2008: Srodki ochrony roslin zalecane do stosowania w lesnictwie w roku 2009. Instytut Badawczy Leśnictwa, analizy i raporty, 11: 1-68.

Grégoire J.-C. & Evans H. F. 2004: Damage and control of BAWBILT organisms, an overview. 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. Kluwer Academic Publishers, Dordrecht, pp. 19-37.

Grégoire J.-C., Merlin J., Pasteels J. M., Jaffuels R., Vouland G. & Schvester D. 1985: Biocontrol of *Dendroctonus micans* by *Rhizophagus grandis* in the Massif Central (France): a first appraisal of the mass-rearing and release methods. Zeitschrift für Angewandte Entomologie, 99: 182-190.

Grodzki W. 1997: Changes in the occurrence of bark beetles on Norway spruce in a forest decline area in the Sudety Mountains in Poland. In: Grégoire J.-C., Liebhold A. M., Stephen F. M., Day K. R. & Salom S. M. (eds.) Proceedings: Integrating cultural tactics into the management of bark beetle and reforestation pests. USDA, Forest Service General Technical Report, NE-236, pp. 105-111.

Grodzki W. 1997: Possibilities of the control of the double-spined bark beetle *Ips duplicatus* C. R. Sahlb in the Southern Poland. Sylwan, 11: 25-36.

Grodzki W. 2003: Distribution range of the double spined bark beetle *Ips duplicatus* C. R Sahlb (Col.: Scolytidae) in the mountain areas of southern Poland. Sylwan 8: 29-36.

Grodzki W. 2004: Zagrozenie górskich drzewostanów swierkowych w zachodniej czésci Beskidów ze strony szkodników owadzich. Leśne Prace Badawcze, 2: 35-47.

Grodzki W. 2008: *Ips cembrae* Heer. (Col.: Curculionidae, Scolytinae) in young larch stands – a new problem in Poland. Forstschutz Aktuell, 44: 8-9.

Grodzki W. 2009: Entomofauna of dying young spruces *Picea abies* (L.) Karst. in the area after forest decline in the Izerskie Mountains. Folia Forestalia Polonica, 51: 161-170.

Grodzki W. 2009: Kornik modrzewiowiec *Ips cembrae* (Heer) (Coleoptera, Curculionidae, Scolytinae) w drzewostanach modrzewiowych południowej Polski. Leśne Prace Badawcze, 70, 355-361.

Grodzki W. 2010: Possibility of the reduction of *Ips cembrae* (Heer) (Coleoptera, Curculionidae, Scolytinae) populations in larch stands in southern Poland. Sylwan, 154: 160-167.

Grodzki W., Kosibowicz M. 2009: Materiały do poznania biologii kornika modrzewiowca *Ips cembrae* (Heer) (Col., Curculionidae, Scolytinae) w warunkach południowej Polski. Sylwan, 153: 587-593.

Grodzki W., Kosibowicz M. & Maczka T. 2008: Skuteczność wystawiania pułapek feromonowych na kornika drukarza *Ips typographus* (L.) w sąsiedztwie wiatrowałów i wiatrołomów. Leśne Prace Badawcze, 69: 365-370.

Grodzki W., Loch J. & Armatys P. 2006: Występowanie kornika drukarza *Ips typographus* L. w uszkodzonych przez wiatr drzewostanach świerkowych masywu Kudłonia w Gorczańskim Parku Narodowym. Ochrona Beskidów Zachodnich, 1: 125-137.

Haidler B. 1998: Pathogene und Parasiten von Fichtenborkenkäfern in einem Fichtenbestand am Achner Kogel bei Tamsweg. Diploma thesis, Formal- und Naturwissenschaftliche Fakultät der Universität Wien, Wien, 144 pp.

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. Insect Pathogens and Insect Parasitic Nematodes. IOBC wprs Bulletin, 26: 257-260.

Hajek A. E. & St. Leger R. J. 1994: Interactions between fungal pathogens and insect hosts. Annual Review of Entomology, 39: 293-322.

Händel U., Kenis M. & Wegensteiner R. 2001: Untersuchungen zum Vorkommen von Pathogenen und Parasiten in Populationen überwinternder Fichtenborkenkäfer (Col., Scolytidae). Mitteilungen der Deutschen Gesellschaft für Allgemeine und Angewandte Entomologie, 13: 423-428.

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.

Hayman J. R., Hayes S. F., Amon J. & Nash T. E. 2001: Developmental expression of two spore wall proteins during maturation of the microsporidian *Encephalitozoon intestinalis*. Infection and Immunity, 69: 7057-7066.

Heliövaara K., Väisänen R. & Immonen A. 1991: Quantitative biogeography of bark beetles (Coleoptera, Scolytidae) in Northern Europe. Acta Forestalia Fennica, 35 pp.

Hellrigl K. 1985: Über Borkenkäfer (Col., Scolytidae) in Zweigen der Zirbe (*Pinus cembra*) in Sudtirol. Anzeiger für Schädlingskunde, Pflanzenschutz und Umweltschutz, 58: 108-110.

Holuša J. & Liška J. 2002: Hypotéza chřadnutí a odumírání smrkových porostů ve Slezsku (Česká republika). Zprávy Lesnického Výzkumu, 47: 9-15.

Holuša J., Lubojacký J. & Knížek M. 2010: Distribution of double-spined spruce bark beetle *Ips duplicatus* in the Czech Republic: spreading in 1997–2009. Phytoparasitica, 38: 435-443.

Holuša J., Lukášová K., Grodzki W., Kula E. & Matoušek P. 2012: Is *Ips amitinus* (Coleoptera: Curculionidae) abundant in wide range of altitudes? Acta Zoologica Bulgarica, in press.

Holuša J., Lukášová K., Wegensteiner R., Grodzki W., Pernek M. & Weiser J. 2012: Pathogens of the bark beetle *Ips cembrae*: microsporidia and gregarines known from other *Ips* species. Journal of Applied Entomology, in press.

Holuša J. & Weiser J. 2005: Biologické postupy boje s lesními škůdci. Zpravodaj Ochrany Lesa, 11: 18-23.

Holuša J., Weiser J. & Drápela K. 2007: Pathogens of *Ips duplicatus* (Coleoptera, Scolytidae) in three areas in Central Europe. Acta Protozoologica, 46: 157-167.

Holuša J., Weiser J. & Pultar O. 2004: Possibility of using of virus against bark beetles? Experiences with simulated infection of two bark beetles, *Ips typographus* and *Ips duplicatus*, with three pathogens. In: Csóka G., Hirka A. & Koltay A. (eds.): Biotic damage in forests. Proceedings of the IUFRO Symposium (WP 7. 03. 10 "Methodology of forest pest and disease survey in Central Europe"). Mátrafüred, Hungary, 12-16 September 2004, pp. 72-78.

Holuša J., Weiser J. & Žižka Z. 2009: Pathogens of the spruce bark beetles *Ips typographus* and *Ips duplicatus*. Central European Journal of Biology, 4: 567-573.

Holuša J., Zahradník P., Knížek M. & Drápela K. 2003: Seasonal flight activity of the double-spined spruce bark-beetle *Ips duplicatus* (Coleoptera, Curculionidae, Scolytinae) in Silesia (Czech Republic). Biologia, 58: 935-941.

Hutka D. 2006: Nowe oblicze kornika modrzewiowca. Trybuna Leśnika, 4: 10-11.

Jakovlev J. & Siitonen J. 2005: Assessment of pest risk involved in timber import from northwestern Russia. In: Rautapää J. & Pouttu A. (eds.) Health status of forests in Finland and Russian Federation, Seminar 2005, 48 pp.

Jakuš R. 1998: Patch level variation on bark beetle attack (Col., Scolytidae) on snapped and uprooted trees in Norway spruce primeval natural forest in endemic conditions: effects of host and insolation. Journal of Applied Entomology, 122: 409-421.

Jankowiak R., Rossa R. & Miśta K. 2007: Survey of fungal species vectored by *Ips cembrae* to European larch trees in Raciborskie forests (Poland). Czech Mycology, 59: 227-239.

Jarošík V. 2005: Růst a regulace populací. Academia, Praha. 170 pp.

Jonsell M., Nordlander G. & Jonsson M. 1999: Colonization patterns of insects breeding in wood-decaying fungi. Journal of Insect Conservation, 3: 145-161.

Jung P., Rohde M. & Lunderstadt J. 1994: Induzierte Resistenz im Leitgewebe der Europaischen Larche *Larix decidua* Mill nach Befall durch den Grossen Larchenborkenkäfer *Ips cembrae* Heer (Col.: Scol.). Journal of Applied Entomology, 117: 427-433.

Jurc M. & Bojović S. 2004: Bark beetle outbreaks during the last decade with special regard to the eight-toothed bark beetle (*Ips amitinus* Eichh.) outbreak in the Alpine region of Slovenia. In: Csóka G., Hirka A. & Koltay A. (eds.) Proceedings of the IUFRO Symposium (WP 7. 03. 10 "Methodology of forest pest and disease survey in Central Europe"). Mátrafüred, Hungary, 12-16 September, 2004, pp. 85-95.

Kaya H. K. 1984: Nematode parasites of bark beetles. In: Nickle W. R. (ed.) Plant and Insect Nematodes. Marcel Dekker, Inc, New York, pp. 727-754.

Kaya H. K. & Gaugler R. 1993: Entomopathogenic nematodes. Annual Review of Entomology, 38: 181-206.

Kenis M., Wermelinger B., Grégoire J.-C. 2004: Research on parasitoids and predators of Scolytidae - a review. 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. Kluwer Academic Publishers, Dordrecht, pp. 237-290.

Kereselidze M. & Wegensteiner R. 2007: Occurrence of pathogens and parasites in *Ips typographus* L. from spruce stands (*Picea orientalis* L.) in Georgia. IOBC wprs Bulletin, 30: 207-210.

Kereselidze M., Wegensteiner R., Goginashvili N., Tvaradze M. & Pilarska D. 2010: Further studies on the occurrence of natural enemies of *Ips typographus* (Coleoptera: Curculionidae: Scolytinae) in Georgia. Acta Zoologica Bulgarica, 62: 131-139.

Kirchhoff J.-F. & Führer E. 1990: Experimentelle Analyse der Infektion und des Entwicklungszyklus von *Malamoeba scolyti* in *Dryocoetes autographus* (Coleoptera: Scolytidae). Entomophaga, 35: 537-544.

Kirisits T., Grubelnik R. & Führer E. 2000: Die ökologische Bedeutung von Bläuepilzen für rindenbrütende Borkenkäfer. FBVA Berichte, 111: 117-137.

Knížek M. 2001: Podkorní hmyz. In: Kapitola P. & Knížek M. (eds.) Výskyt lesních škodlivých činitelů v roce 2000 a jejich očekávaný stav v roce 2001. Zpravodaj Ochrany Lesa (Suppl.), 76: 17-27.

Knížek M. 2001: Progradation of “small spruce bark beetle species”. Journal of Forest Science, 47: 113-114.

Knížek M. 2002: Podkorní hmyz. In: Kapitola P. & Knížek M. (eds.) Výskyt lesních škodlivých činitelů v roce 2001 a jejich očekávaný stav v roce 2002. Zpravodaj Ochrany Lesa (Suppl.), 68: 15-24.

Knížek M. 2003: Podkorní hmyz. In: Kapitola P. & Knížek M. (eds.) Výskyt lesních škodlivých činitelů v roce 2002 a jejich očekávaný stav v roce 2003. Zpravodaj Ochrany Lesa (Suppl.), 64: 15-24.

Knížek M. 2005: Podkorní hmyz. In: Kapitola P. & Baňař P. (eds.) Výskyt lesních škodlivých činitelů v roce 2004 a jejich očekávaný stav v roce 2005. Zpravodaj Ochrany Lesa (Suppl.), 72: 18-26.

Knížek M. 2006: Lýkožout modřinový *Ips cembrae* (Heer). Lesnická Práce, 12: Appendix, I-IV.

Knížek M. 2008: Podkorní hmyz. In: Knížek M. & Pešková V. (eds.) Výskyt lesních škodlivých činitelů v roce 2007 a jejich očekávaný stav v roce 2008. Zpravodaj Ochrany Lesa (Suppl.), 74: 21-33.

Knížek M. 2009: Podkorní hmyz. In: Knížek M. (ed.) Výskyt lesních škodlivých činitelů v roce 2008 a jejich očekávaný stav v roce 2009. Zpravodaj Ochrany Lesa (Suppl.), 72: 20-31.

Knížek M. 2010: Monitoring lýkožrouta severského v Česku v roce 2009. Lesnická Práce, 89: 46-47.

Knížek M. & Holuša J. 2006: Podkorní hmyz. In: Kapitola P. (ed.), Výskyt lesních škodlivých činitelů v roce 2005 a jejich očekávaný stav v roce 2006. Zpravodaj Ochrany Lesa (Suppl.), 76: 20-31.

Knížek M. & Holuša J. 2007: Podkorní hmyz. In: Knížek M. (ed.) Výskyt lesních škodlivých činitelů v roce 2006 a jejich očekávaný stav v roce 2007. Zpravodaj Ochrany Lesa (Suppl.), 74: 21-32.

Knížek M. & Trýzna M. 2002: Kůrovci na borovici vejmutovce v Národním parku České Švýcarsko. Zpravodaj Ochrany Lesa, 8: 54-55.

Knížek M. & Zahradník P. 1996: Mass outbreak of *Ips duplicatus* Sahlberg (Coleoptera, Scolytidae). XX. International Congress of Entomology – Proceedings, Italy, 25-31 August, Firenze, pp. 527.

Knížek M. & Zahradník P. 2004: Podkorní hmyz. In: Kapitola P., Knížek M. & Baňař P. (eds.) Výskyt lesních škodlivých činitelů v roce 2003 a jejich očekávaný stav v roce 2004. Zpravodaj Ochrany Lesa (Suppl.), 80: 30-39.

Koehler P. G., Patterson R. S. & Martin W. R. 1992: Susceptibility of cockroaches (Dictyoptera: Blattellidae, Blattidae) to infection by *Steinernema carpocapsae*. Journal of Economic Entomology, 85: 1184-1187.

Kohnle U., Vité J. P., Erbacher C., Bartels J. & Francke W. 1988: Aggregation response of European engraver beetles of the genus *Ips* mediated by terpenoid pheromones. Entomologia Experimentalis et Applicata, 49: 43-53.

Koponen M. 1975: Distribution of *Ips amitinus* in Finland in 1950-1973. Annales Entomologici Fennici, 41: 65-69.

Krehan H. 2004: Data sheet: *Ips cembrae* (Großer Lärchenborkenkäfer). Forstschutz Aktuell, 32: 9.

Krehan H. & Cech T. L. 2004: Larch damage in Upper Styria. An example of the complex effects of damage agents. Forstschutz Aktuell, 32: 4-8.

Krehan H. & Steyer G. 2005: Borkenkäfer-Monitoring und Borkenkäfer-kalamität 2004. Forstschutz Aktuell, 33: 12-14.

Kreutz J., Vaupel O. & Zimmermann G. 2004: 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.

Kula E. & Ząbecki W. 2001: Attractiveness of spruce for cambioxylophages as related to stand age. Journal of Forest Science, 47: 88-96.

Kula E. & Ząbecki W. 2002: Struktura kambioxylofágní fauny smrku rezervace Kněhyně a hospodářských smrkových porostů v Beskydech. Časopis Slezského Muzea Opava (A), 51: 155-164.

Kula E. & Ząbecki W. 2010: Creation of the merocoenosis of bark beetles (Scolytidae) of Norway spruce (*Picea abies* /L./ Karst.) depending on damage to trees and gradation conditions. Journal of Forest Science, 56: 474-484.

Kula E., Kajfosz, R. & Polívka J. 2009: Cambioxylophagous fauna of blue spruce (*Picea pungens* Engelmann) in the Krušné hory Mts. (Czech Republic). Beskydy, 2: 149-156.

Kula E., Kajfosz R. & Polívka J. 2011: Cambioxylophagous fauna developing on logging residues of blue spruce (*Picea pungens* Engelmann). Journal of Forest Science, 57: 24-33.

Kula E., Kajfosz R. & Ząbecki W. 2007: Cambioxylophagous fauna of young spruce stands damaged by snow in the Beskids. Journal of Forest Science, 53: 413-423.

Kuś J. & Kuś D. 2004: Entomofauna rozkładającego się drewna świerka, buka i brzozy na terenie Karkonoskiego Parku Narodowego. In: Štursa J., Mazurski K. R., Palucki A. & Potocka J. (eds.) Geoekologické problémy Krkonoš. Sborník Mezinárodní Vědecké Konference, Listopad 2003, Szklarska Poręba. Opera Corcontica, 41: 269-280.

Landa Z., Horňák P., Osborne L. S., 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.

Landa Z., Křenová Z. & Vojtěch O. 2007: Využití houby *Beauveria bassiana* v ochraně proti lýkožroutu smrkovému. Lesnická práce, 10: 14-15.

Lange C. E. & Wittenstein E. 2002: The life cycle of *Gregarina ronderosi* n. sp. (Apicomplexa: Gregarinidae) in the Argentine grasshopper *Dichroplus elongatus* (Orthoptera: Acrididae). Journal of Invertebrate Pathology, 79: 27-36.

Lieutier F. 1980: Le parasitisme d'*Ips sexdentatus* (Boern) (Coleoptera: Scolytidae) par les nématodes du genre *Parasitaphelenchus* Fuchs. Relations avec le parasitisme par *Contortylenchus diplogaster* (v. Lins.). Revue Nématology, 3: 271-281.

Lieutier F. 2002: Mechanisms of resistance in conifers and bark beetle attack strategies. In: Wagner M. R., Clancy K. M., Lieutier F. & Paine T. D. (eds.) Mechanisms and deployment of resistance in trees to insects. Kluwer Academic Publishers, Dordrecht, pp. 31-77.

Lipa J. J. 1967: Studies on gregarines (Gregarinomorpha) of arthropods in Poland. Acta Protozoologica, 5: 97-179.

Locklin J. L. & Vodopich D. S. 2010: Patterns of gregarine parasitism in dragonflies: host, habitat, and seasonality. Parasitology Research, 107: 75-87.

Luitjes J. 1974: *Ips cembrae*, a new noxious forest insect in the Netherlands. Nederlands Bosbouw Tijdschrift, 46: 244-246.

Lukášová K. & Holuša J. 2011: *Gregarina typographi* (Eugregarinorida: Gregarinidae) in the bark beetle *Ips typographus* (Coleoptera: Curculionidae): changes in infection level in the breeding system. Acta Protozoologica, 50: 311-318.

MacDougall M. M. 1942: A study of temperature effects on gregarines of *Tenebrio molitor* larvae. Journal of Parasitology, 28: 233-240.

Mandelshtam M. J. 1999: Current status of *Ips amitinus* Eichh. (Coleoptera, Scolytidae) in North-West Russia. Entomologica Fennica, 10: 29-34.

Mazur A., Łabędzki A. & Raj A. 2006: Observations of phenology and frequencing of the spruce bark beetle (*Ips typographus* L.) and its accompanying species in the uppermost subalpine spruce forest zone environment in the Karkonoski National Park. In: Kolk A. (ed.) Insect outbreaks in managed and unmanaged forests. Instytut Badawczy Leśnictwa, Warszawa, pp. 29-48.

Mihalciuc V., Danci A., Lupu D. & Olenici N. 2001: Situation of the main bark and wood boring insects which damaged conifer stands in the last 10 years in Romania. Anale Institutul de Cercetari si Amenjari Silvice, 1: 48-53.

Michalková V., Krascsenitsová E. & Kozánek M. 2011: On the pathogens of the spruce bark beetle *Ips typographus* (Coleoptera: Scolytinae) in the Western Carpathians. Biologia, 67: 217-221.

Michalski J. & Mazur A. 1999: Korniki. Praktyczny przewodnik dla leśników. Wydawnictwo Świat, Warszawa, 188 pp.

Mráček Z. 1986: Nematodes and other factors controlling the sawfly, *Cephaleia abietis* (Pamphilidae, Hymenoptera), in Czechoslovakia. Forest Ecology and Management, 15: 75-79.

Mráček Z. & Bečvář S. 2000: Insect aggregations and entomopathogenic nematode occurrence. Nematology, 2: 297-301.

Netherer S., Pennerstorfer J., Baier P., Führer E. & Schopf A. 2003: Monitoring and risk assessment of the spruce bark beetle, *Ips typographus*. In: McManus M. L. & Liebhold A. M. (eds.) Proceedings: Ecology, Survey and Management of Forest Insects. Krakow, Poland, Gen. Tech. Rep., NE-311, Dept. of Agriculture, Forest Service, Northeastern Research Station, Newtown Square, PA: U.S., 1-5 September, 2002, pp. 75-79.

Novotný J., Grodzki W., Knížek M., McManus M. & Turčáni M. 2002: The impact of spruce bark beetle populations on mountain spruce forests and ecological approaches to their management. Proceedings of the NATO Advanced Research Workshop, Stará Lesná, Slovakia, IOS Press, Amsterdam, Netherlands, 22-26 May, 2002, pp. 250-258.

OEPP/EPPO 2005: *Ips cembrae* and *Ips subelongatus*. Bulletin OEPP/EPPO, 35: 445-449.

Økland B. & Skarpaas O. 2008: Draft pest risk assessment report on the small spruce bark beetle, *Ips amitinus*. Commissioned report from Norwegian Forest and Landscape Institute, 20 pp.

Omoto C. K., Toso M., Tang K. & Sibley L. D. 2004: Expressed sequence tag (EST) analysis of gregarine gametocyst development. International Journal for Parasitology, 34: 1265-1271.

Pavlin R. 2001: The catch of the larch bark beetle *Ips cembrae* (Heer) with pheromone traps in Slovenia. Journal of Forest Science, 47 (Special Issue 2): 143-146.

Pavlovskij, E. N. 1955: Vrediteli lesa spravochnik. Izd-vo Akademii Nauk SSSR, Moskva, 1097 pp.

Perkins F. O. 2000: Order Neogregarinorida Grassé, 1953. In: Lee J. J., Leedale G. F. & Bradbury P. (eds.) The illustrated guide to the Protozoa, 2nd ed. Society of Protozoologists, Lawrence, pp. 288-298, pp. 353-369.

Pettersson E. M. 2001: Volatiles from potential host of *Rhopalicus tutela* a bark beetle parasitoid. Journal of Chemical Ecology, 27: 2219-2217.

Peuvel-Fanget I., Polonais V., Brosson D., Texier C., Kuhn L., Peyret P., Vivares C. P. & Delbac F. 2006: EnP1 and EnP2, two proteins associated with the *Encephalitozoon cuniculi* endospore, the chitin-rich inner layer of the microsporidian spore wall. International Journal for Parasitology, 36: 309-318.

Pfeffer A. 1955: Fauna ČSR, Kůrovci – Scolytoidea. Nakladatelství ČAV, Praha, 344 pp.

Pfeffer A. 1989: Kůrovcovití Scolytidae a jádrohlodovití Platypodidae. Academia, Praha, 138 pp.

Pfeffer A. 1995: Zentral- und westpaläarktische Borken-und Kernkäfer (Coleoptera: Scolytidae, Platypodidae). Pro Entomologia, Basel, 310 pp.

Pfeffer A. & Knížek M. 1995: Expanze lýkožrouta *Ips duplicatus* (Sahlb.) ze severské tajgy. Zpravodaj Ochrany Lesa, 2: 8-11.

Pfeffer A. & Skuhravý V. 1995: Der Buchdrucker (*Ips typographus* L.) (Col. Scolytidae) und seine Problematik in der Tschechischen Republik. Anzeiger für Schädlingskunde, Pflanzenschutz, Umweltschutz, 68: 151-152.

Pfeil W. 1827: Über Insektenschaden in den Wäldern, die Mittel ihm vorzubeugen und seine Nachteile zu vermindern. Verlag Boicke, Berlin, 72 pp.

Phelps N. B. D. & Goodwin A. E. 2008: Vertical transmission of *Ovipleistophora ovariae* (Microspora) within the eggs of the Golden Shiner. Journal of Aquatic Animal Health, 20: 45-53.

Plašil P. & Cudlín P. 2005: Population dynamics of eight-toothed spruce bark beetle (*Ips typographus* [L.]) in the area of National Nature Reserve Praděd in 1998–2001. Journal of Forest Science, 51: 359-370.

Poinar G. O. & Deschamps N. 1981: Susceptibility of *Scolytus multistriatus* to neoaplectanid and heterorhabditid nematodes. Environmental Entomology, 10: 85-87.

Pop M., Kula E., Maňas P. & Kajfosz R. 2010: Chemical thinning in blue spruce (*Picea pungens* Engelm.) stands and its effects on cambioxylophagous fauna. Journal of Forest Science, 56: 225-235.

Postner M. 1974: *Ips cembrae*. In: Schwenke W. (ed.) Die Forstschädlinge Europas. II. Band. Käfer. Paul Parey, Hamburg, pp. 458-459.

Pultar O. & Weiser J. 2004: Výsledky posledních patologických studií dominantních kůrovců v NP Šumava a jejich využití. In: Sborník referátů. 28. setkaní lesníků tří generací na téma "Nebezpečí kůrovce v roce 2004, Praha-Novotného lávka, Česká lesnická společnost, VÚLHM Jíloviště-Strnady, Lesnická práce, 19 March 2004, pp. 82-88.

Purrini K. 1977: Über eine neue Schizogregarinen-Krankheit der Gattung *Mattesia* Naville (Sporozoa, Dischizae) des Zottigen Fichtenborkenkäfers, *Dryocoetes autographus* Ratz (Coleoptera, Scolytidae). Anzeiger für Schädlingskunde, Pflanzenschutz, Umweltschutz, 50, 132-135.

Purrini K. 1978: Protozoen als Krankheitserreger bei einigen Borkenkäferarten (Col., Scolytidae) im Königsee-Gebiet, Oberbayern. Anzeiger für Schädlingskunde, Pflanzenschutz, Umweltschutz, 51: 171-175.

Purrini K. & Führer E. 1979: Experimentelle Infection von *Pityogenes chalcographus* L. (Coleoptera, Scolytidae) durch *Malamoeba scolyti* Purrini (Amoebina, Amoebidae) und *Menzbieria chalcographi* Weiser (Neogregarina, Ophryocystidae). Anzeiger für Schädlingskunde, Pflanzenschutz und Umweltschutz, 52: 167-173.

Purrini K. & Žižka Z. 1983: More on the life cycle of *Malamoeba scolyti* (Amoebidae: Sarcomastigophora) parasitizing the bark beetle *Dryocoetes autographus* (Scolytidae, Coleoptera). Journal of Invertebrate Pathology, 42: 96-105.

Ramos-Rodríguez O., Campbell J. F. & Ramaswamy S. B. 2006: Pathogenicity of three species of entomopathogenic nematodes to some major stored-product insect pests. Journal of Stored Products Research, 42: 241-252.

Rebenstorff H. & Francke W. 1982: The large larch bark beetle: monitoring with attractants? Allgemeine Forst Zeitschrift, 37: 450.

Redfern D. B. 1989: The roles of the bark beetle *Ips cembrae*, the woodwasp *Urocerus gigas* and associated fungi in dieback and death of larches. In: Wilding N., Collins N. M., Hammond P. M. & Webber J. F. (eds.) Insect fungus interactions, 14th symposium of the Royal Entomological Society of London in collaboration with the British Mycological Society. Academic Press, London, pp. 195-204.

Redhead S. A., Kirk P. M., Keeling P. J. & Weiss L. M. 2009: Proposals to exclude the phylum Microsporidia from the Code. Mycotaxon, 108: 505-507.

Roberts D. W. & Hajek A. E. 1992: Entomopathogenic fungi as bioinsecticides. In: Leatham G. F. (ed.) Frontiers of industrial mycology. Chapman and Hall, New York, pp. 144-159.

Rohde M. 1995: Physiological investigations into susceptibility, defence reactions and resistance, in relations between larch and the larch bark beetle (*Ips cembrae*). Mitteilungen der Deutschen Gesellschaft für allgemeine und angewandte Entomologie, 10: 51-54.

Rohde M., Waldmann R. & Lunderstandt J. 1996: Induced defence reaction in the phloem of spruce (*Picea abies*) and larch (*Larix decidua*) after attack by *Ips typographus* and *Ips cembrae*. Forest Ecology and Management, 86: 51-59.

Rühm W. 1956: Die Nematoden der Ipiden: Parasitologische Schriftenreihe, 6., Veb Gustav, Fischer Verlag, Jena, 437 pp.

Shannag H. K. & Capinera J. L. 2000: Interference of *Steinernema carpocapsae* (Nematoda: Steinernematidae) with *Cardiochiles diaphaniae* (Hymenoptera: Braconidae), a parasitoid of melonworm and pickleworm (Lepidoptera: Pyralidae). Environmental Entomology, 29: 612-617.

Shapiro D. I. & McCoy C. W. 2000: Virulence of entomopathogenic nematodes to *Diaprepes abbreviatus* (Coleoptera: Curculionidae) in the laboratory. Journal of Economic Entomology, 93: 1090-1095.

Schawang J. E. & Janovy J. Jr. 2001: The response of *Gregarina niphandrodes* (Apicomplexa: Eugregarinida: Septatina) to host starvation in *Tenebrio molitor* (Coleoptera: Tenebrionidae) adults. Journal of Parasitology, 87: 600-605.

Schneider H. J. 1977: Experience in the control of the large larch bark beetle in stands of low vitality. Allgemeine Forst Zeitschrift, 32: 1115-1116.

Sienkiewicz P. & Lipa J. 2008: *Gregarina vizri* Lipa, 1968 (Apicomplexa: Eugregarinida) recorded in Poland in an expansive plant pest the cereal ground beetle *Zabrus tenebrioides* (Goeze) (Coleoptera: Carabidae). Journal of Plant Protection Research, 48: 189-193.

Sierpiński Z. 1958: Zagadnienie zwalczania kornika zrosłozębnego (*Ips duplicatus* Sahlb.). Sylwan, 102: 68-75.

Simmons L. W. 1990: Post-copulatory guarding female choice and the levels of gregarine infections in the field cricket *Gryllus binzaculatus*. Behavioral Ecology and Sociobiology, 26: 403-407.

Smith A. J. & Cook T. J. 2008: Host specificity of five species of Eugregarinida among six species of cockroaches (Insecta: Blattodea). Comparative Parasitology, 75: 288-291.

Smith A. J., Cook T. J. & Lutterschmidt W. I. 2007: Effects of temperature on the development of *Gregarina cubensis* (Apicomplexa: Eugregarinida) parasitizing *Blaberus discoidalis* (Blattaria: Blaberidae). Journal of Parasitology, 93: 583-588.

Stauffer C., Kirisits T., Nussbaumer C., Pavlin R. & Wingfield M. J. 2001: Phylogenetic relationships between the European and Asian eight spined larch bark beetle populations (Coleoptera, Scolytidae) inferred from DNA sequences and fungal associates. EUROPEAN Journal of Entomology, 98: 99-105.

Stauffer C. & Zuber M. 1998: *Ips amitinus* var. montana (Coleoptera, Scolytidae) is synonymous to *Ips amitinus*: a morphological, behavioural and genetic re-examination. Biochemical Systematics and Ecology, 26: 171-183.

Steinhaus E. A. 1956: Microbial control: the emergence of an idea. Hilgardia, 26: 107-160.

Stoakley J. T., Bakke A., Renwick J. A. A. & Vité J. P. 1978: The aggregation pheromone system of the larch bark beetle, *Ips cembrae* Heer. Zeitschrift für Angewandte Entomologie, 86: 174-177.

Stratmann J. 2004: Borkenkäferkalamitt 2003 was haben wir gelernt, sind wir für 2004 gerüstet? Forst Holz, 59: 166-169.

Suzuki S. & Imada H. 1993: Effect of temperatures on the developmental period of *Ips cembrae* (Heer) (Coleoptera: Scolytidae). Journal of the Japanese Forest Society, 75: 538-540.

Šrot M. 1976: Některé nové poznatky o zakládání sesterského pokolení lýkožrouta modřínového (*Ips cembrae* Heer.) v ČSR. Lesnictví, 22: 979-996.

Takov D., Doychev D., Linde A., Draganova S. & Pilarska D. 2011: Pathogens of bark beetles (Coleoptera: Curculionidae) in Bulgarian forests. Phytoparasitica, 39: 343-352.

Takov D., Doychev D., Wegensteiner R. & Pilarska D. 2007: Study on the pathogens of bark beetles (Coleoptera, Scolytidae) from different coniferous stands in Bulgaria. Acta Zoologica Bulgarica, 59: 87-96.

Takov D., Pilarska D. & Wegensteiner R. 2006: Entomopathogens in *Ips typographus* (Coleoptera: Scolytidae) from several spruce stands in Bulgaria. Acta Zoologica Bulgarica, 58: 409-420.

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.

Tanada Y. & Kaya H. K. 1993: Protozoan infection: Apicomplexa, Microspora. In: Tanada Y. & Kaya H. K. (eds.) Insect Pathology. Academic Press, San Diego, pp. 414-458.

Terasaki, Y., Yosida N., Fukuyama K. & Furuta K. 1987: Response of *Larix leptolepis* to inoculated *Ips cembrae*. Bulletin of the Tokyo University Forests, 77: 19-30.

Théodoridés J. 1984: The phylogeny of the Gregarinia (Sporozoa). Origins of Life and Evolution of Biospheres, 13: 339-342.

Thomas F., Oget E., Gente P., Desmots D. & Renaud F. 1999: Assortative pairing with respect to parasite load in the beetle *Timarcha maritima* (Chrysomelidae). Journal of Evolutionary Biology, 12: 385-390.

Thong C. H. S. & Webster J. M. 1975: Effects of bark beetle nematode, *Contortylenchus reversus*, on gallery construction, fecundity, and egg viability of douglas-fir beetle, *Dendroctonus pseudotsugae* (Coleoptera-Scolytidae). Journal of Invertebrate Pathology, 26: 235-238.

Tonka T., Weiser J. Jr. & Weiser J. 2010: Budding: A new stage in the development of *Chytridiopsis typographi* (Zygomycetes: Microsporidia). Journal of Invertebrate Pathology, 104: 17-22.

Toso M. & Omoto C. K. 2007: Ultrastructure of *Gregarina niphandrodes* nucleus through stages from unassociated trophozoites to gamonts in syzygy and the syzygy junction. Journal of Parasitology, 93: 479-484.

Tronchin G. & Schrével J. 1977: Chronologie des modifications ultrastructurales au cours de la croissance de *Gregarina blaberae*. Journal of Protozoology, 24: 67-82.

Turčáni M. & Hlásny T. 2007: Spatial distribution of four spruce bark beetles in north-western Slovakia. Journal of Forest Science, 53: 45-52.

Ulyshen M. D. & Pucci T. M. 2010: The importace of forest type, tree species and wood posture to saproxylic wasp (Hymenoptera) communities in the southeastern United States. Journal of Insect Conservation, 10: 5-10.

Unal S., Yaman M., Tosun O. & Aydin C. 2009: Occurence of *Gregarina typographi* (Apicomplexa, Gregarinidae) and *Metschnikowia typographi* (Ascomycota, Metschnikowiaceae) in *Ips sexdentatus* (Coleoptera: Curculionidae, Scolytinae) populations in Kastamonu (Turkey). Journal of Animal and Veterinary Advances, 8: 2687-2691.

Valigurová A., Michalková V. & Koudela B. 2009: Eugregarine trophozoite detachment from the host epithelium via epimerite retraction: Fiction or fact? International Journal for Parasitology, 39: 1235-1242.

Vanninen I. 1996: Distribution and occurence of four entomopathogenic fungi in Finland: effect of geographical location, habitat types and soil type. Mycological Research, 100: 93-101.

Vávra J. & Larsson J. I. R. 1999: Structure of the microsporidia. In: Whittner M. & Weiss L. M. (eds.) The microsporidia and microsporidiosis. ASM Press, Washington DC, pp. 7-84.

Voolma K., Mandelshtam M. J., Shcherbakov A. N., Yakovlev E. B., Ounap H., Süda I., Popovichev B. G., Sharapa T. V., Galasjeva T. V., Khairetdinov R. R., Lipatkin V. A. & Mozolevskaya E. G. 2004: Distribution and spread of bark beetles (Coleoptera: Scolytidae) around the Gulf of Finland: a comparative study with notes on rare species of Estonia, Finland and North-Western Russia. Entomologica Fennica, 15: 198-210.

Watzek G. & Niemeyer H. 1996: Verminderung der Borkenkäfergefähr durch Harvestertechnik und Arbeitsorganisation. Forst Holz, 51: 247-250.

Wegensteiner R. 1994: *Chytridiopsis typographi* (Protozoa, Microsporidia) and other pathogens in *Ips typographus* (Coleoptera, Scolytidae). IOBC/wprs Bulletin, 17: 39-42.

Wegensteiner R. 1996: Laboratory evaluation of *Beauveria bassiana* (Bals.) Vuill. against *Ips typographus* (Coleoptera, Scolytidae). IOBC/WPRS Bulletin, 19: 186-189.

Wegensteiner R. 2004: Pathogens in bark beetles. 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, Asynthesis. Kluwer Academic Publishers, Dordrecht, pp. 291-313.

Wegensteiner R., Dedryver C.-A. & Pierre J.-S. 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. 1995: A new *Entomopoxvirus* in the bark beetle *Ips typographus* (Coleoptera: Scolytidae). Journal of Invertebrate Pathology, 65: 203-205.

Wegensteiner R. & Weiser J. 1996: Occurrence of *Chytridiopsis typographi* (Microspora, Chytridiopsida) in *Ips typographus* L. (Col., Scolytidae) field population and in a laboratory stock. Journal of Applied Entomology, 120: 595-602.

Wegensteiner R. & Weiser J. 1996: Untersuchungen zum Auftreten von Pathogen by *Ips typographus* (Coleoptera: Scolytidae) aus einem Naturschutzgebiet in Schwartzwald (Baden-Württemberg). Anzeiger für Schädlingskunde, Pflanzenschutz, Umweltschutz, 69: 162-167.

Wegensteiner R. & Weiser J. 2004: Annual variation of pathogen occurrence and pathogen prevalence in *Ips typographus* L. (Col, Scolytidae) from the BOKU University Forest Demonstration Centre. Journal of Pest Science, 77: 221-228.

Wegensteiner R., Weiser J. & Führer E. 1996: Observations on the occurrence of pathogens in the bark beetle *Ips typographus* L. (Coleoptera, Scolytidae). Journal of Applied Entomology, 120: 199-204.

Weiser J 1954: Příspěvek k znalosti cizopasníků kůrovce *Ips typographus* L. Věstník Československé Společnosti Zoologické, 18: 217-224.

Weiser J. 1955: Příspěvek k znalosti cizopasníků kůrovce *Ips typographus* L. II (Contributions to the knowledge of *Ips typographus* parasites, II.). Věstník Československé Společnosti Zoologické, 19: 374-380.

Weiser J. 1966: Nemoci hmyzu. Academia, Praha, 556 pp.

Weiser J. 1970: Three new pathogens of the douglas fir beetle *Dendroctonus pseudotsugae*: *Nosema dendroctoni* n.sp., *Ophryocystis dendroctoni* n.sp., and *Chytridiopsis typographi* n.comb. Journal of Invertebrate Pathology, 16: 436-441.

Weiser J. 2002: Patogenní organismy. In: Skuhravý V. (ed.) Lýkožrout smrkový (*Ips typographus* L.) a jeho kalamity. Agrospoj, Praha, pp. 97-100.

Weiser J. & Briggs J. D. 1971: Identification of pathogens. In: Burges H. D. & Hussey N. W. (eds.) Microbial control of insects and mites. Academic Press, London, pp. 13-66.

Weiser J., Holuša J. & Žižka Z. 2006: *Larssoniella duplicati* n.sp. (Microsporidia. Unikaryonidae), a newly described pathogen infecting the double-spined spruce bark beetle, *Ips duplicatus* (Coleoptera, Scolytidae) in the Czech Republic. Journal of Pest Science, 79: 127-135.

Weiser J. & Mráček Z. 1988: Parazitické hlístice hmyzu. Academia, Praha, 260 pp.

Weiser J., Pultar O. & Žižka Z. 2000: Biological protection of forest against bark beetle outbreaks with poxvirus and other pathogens. IUAPPA, Section B, 12: 168-172.

Weiser J. & Wegensteiner R. 1994: A new *Entomopoxvirus* in the bark beetle *Ips typographus* (Coleoptera, Scolytidae) in Czechoslovakia. Zeitschrift für Angewandte Zoologie, 80: 425-434.

Weiser J., Wegensteiner R., Händel U. & Žižka Z. 2003: Infections with the Ascomycete *Metschnikowia typographi* n. sp. in the bark beetle *Ips typographus* and *Ips amitinus* (Col., Scolytidae.). Folia Microbiologica, 48: 611-618.

Weiser J., Wegensteiner R. & Žižka Z. 1995: *Canningia spinidentis* gen. et sp. n. (Protista: Microspora), a new pathogen of the fir bark beele *Pityokteines spinidens*. Folia Parasitologica, 42: 1-10.

Weiser J., Wegensteiner R. & Žižka Z. 1998: *Unikaryon montanum* sp.n., (Protista, Microspora), a new pathogen of the spruce bark beetle, *Ips typographus* (Coleoptera: Scolytidae). Folia Parasitologica, 45: 191-195.

Wermelinger B. 2002: Development and distribution of predators and parasitoids during two consecutive years of an *Ips typographus* (Col., Scolytidae) infestation. Journal of Applied Entomology, 126: 521-527.

Wermelinger B. 2004: Ecology and management of the spruce bark beetle *Ips typographus* – a review of recent research. Forest Ecology and Management, 202: 67-82.

Wermelinger B., Rigling A., Schneider Mathis D. & Dobbertin M. 2008: Assessing the role of bark- and wood-boring insects in the decline of Scots pine (*Pinus sylvestris*) in the Swiss Rhone valley. Ecological Entomology, 33: 239-249.

Weslien J. 1992: The arthropod complex associated with *Ips typographus* (L.) (Coleoptera, Scolytidae): species composition, phenology, and impact on bark beetle productivity. Entomologica Fennica, 3: 205-213.

Weslien J., Annila E., Bakke A., Bejer B., Eidmann H. H., Narvestad K., Nikula A. & Ravn H. P. 1989: Estimating risk for spruce bark beetle (*Ips typographus* (L.)) damage using pheromone-baited traps and trees. Scandinavian Journal of Forest Research, 4: 87-98.

Westhuizen van der K., Wingfield M., Yamaoka Y., Kemp G. H. J. & Crous P. W. 1995: A new species of *Ophiostoma* with a *Leptographium* anamorph from larch in Japan. Mycological Research, 99: 1334-1338.

Witrylak M. 2008: Studies of the biology, ecology, phenology, and economic importace of *Ips amitinus* (Eichh.) (Col., Scolytidae) in experimental forests of Krynica (Beskid Sadecki, Southern Poland). Acta Scientiarum Polonorum. Silvarum Colendarum Ratio et Industria Lignaria, 7: 75-92.

Wittner M. & Weiss L. M. 1999: The microsporidia and microsporidiosis. ASM Press, Washington DC, 553 pp.

Xu Y. & Weiss L. M. 2005: The microsporidian polar tube: a highly specialised invasion organelle. International Journal for Parasitology, 35: 941-953.

Yamaguchi T., Sasaki K. & Matsuzaki S. 1989: Reaction of Japanese larch inoculated with *Ceratocystis piceae*. Annual Report of the Hokkaido Research Center, Forestry and Forest Products Research Institute, Hokkaido, pp. 75-79.

Yaman M. 2002: *Gregarina phyllotretae* Hoshide 1953, a protozoan parasite of the flea beetles, *Phyllotreta undulata* and *P. atra* (Coleoptera: Chrysomelidae) in Turkey. Applied Entomology and Zoology, 37: 649-653.

Yaman M. 2007: *Gregarina typographi* Fuchs, a gregarine pathogen of the six-toothed pine bark beetle, *Ips sexdentatus* (Boerner) (Coleoptera: Curculionidae, Scolytinae) in Turkey. Turkish Journal of Zoology, 31: 359-363.

Yaman M. 2008: First results on distribution and occurrence of the insect pathogenic alga *Helicosporidium* sp. (Chlorophyta: Trebouxiophyceae) in the populations of the great spruce bark beetle, *Dendroctonus micans* (Kugelann) (Coleoptera: Curculionidae, Scolytinae). North-Western Journal of Zoology, 4: 99-107.

Yaman M. 2008: On the occurrence of a gregarine parasite from *Psylloides cupreus* Koch 1803 (Coleoptera: Chrysomelidae) of Turkey. North-Western Journal of Zoology, 4: 167-172.

Yaman M. & Baki H. 2011: First Record of *Entomopoxvirus* of *Ips typographus* (Linnaeus) (Coleoptera: Curculionidae, Scolytinae) for Turkey. Acta Zoologica Bulgarica, 63: 199-202.

Yaman M. & Radek R. 2007: Infection of the predator beetle *Rhizophagus grandis* Gyll. (Coleoptera, Rhizophagidae) with the insect pathogenic alga *Helicosporidium* sp. (Chlorophyta: Trebouxiophyceae). Biological Control, 41: 384-388.

Yaman M., Radek R., Aydin C., Tosun O. & Ertürk Ö. 2009: First record of the insect pathogenic alga *Helicosporidium* sp. (Chlorophyta: Trebouxiophyceae) infection in larvae and pupae of *Rhizophagus grandis* Gyll. (Coleoptera, Rhizophaginae) from Turkey. Journal of Invertebrate Pathology, 102: 182-184.

Yamaoka Y., Wingfield M. J., Ohsawa M. & Kuroda Y. 1998: Ophiostomatoid fungi associated with *Ips cembrae* in Japan and their pathogenicity to Japanese larch. Mycoscience, 39: 367-378.

Zahradník P. (ed.) 1996: Výskyt lesních škodlivých činitelů v roce 1995 a jejich očekávaný stav v roce 1996. Zpravodaj Ochrany Lesa (Suppl.), 1996: 1-64.

Zahradník P. (ed.) 1997: Výskyt lesních škodlivých činitelů v roce 1996 a jejich očekávaný stav v roce 1997. Zpravodaj Ochrany Lesa (Suppl.), 1997: 1-88.

Zahradník P. & Knížek M. 1998: Podkorní hmyz. In: Zahradník P. (ed.) Výskyt lesních škodlivých činitelů v roce 1997 a jejich očekávaný stav v roce 1998. Zpravodaj Ochrany Lesa (Suppl.), 1998: 1-88.

Zahradník P. & Knížek M. 1999: Podkorní hmyz. In: Knížek M. & Kapitola P. (eds.) Výskyt lesních škodlivých činitelů v roce 1998 a jejich očekávaný stav v roce 1999. Zpravodaj Ochrany Lesa (Suppl.), 1999: 13-21.

Zahradník P. & Knížek M. 2000: Podkorní hmyz. In: Knížek M., Kapitola P. (eds.) Výskyt lesních škodlivých činitelů v roce 1999 a jejich očekávaný stav v roce 2000. Zpravodaj Ochrany Lesa (Suppl.), 2000: 16-23.

Zasev B. 1952: Merki za borba srestu varhovija korojad (*Ips acuminatus* Gyll.). Nauchnye. Trudy lesotehnitscheskogo Fakulteta selskostopanskoy Akademii, Sofia, 1: 109-120.

Zhang Q. H., Birgersson G., Schlyter F. & Chen-Guo F. 2000: Pheromone components in the larch bark beetle, *Ips cembrae*, from China: quantitative variation among attack phases and individuals. Journal of Chemical Ecology, 26: 841-858.

Zhang Q. H., Byers J. A. & Schlyter F. 1992: Optimal attack density in the larch bark beetle, *Ips cembrae* (Coleoptera: Scolytidae). Journal of Applied Ecology, 29: 672-678.

Zhang Q. H., Schlyter F., Chen-Guo F. & Wang Y. J. 2007: Electrophysiological and behavioral responses of *Ips subelongatus* to semiochemicals from its hosts, non-hosts, and conspecifics in China. Journal of Chemical Ecology, 33: 391-404.

Zitterer P. M. 2002: Antagonists of *Ips acuminatus* (Gyllenhall) with special consideration of pathogens. Diploma thesis. Universität für Bodenkultur, Wien, 56 pp.

Zuber M. 1992: Untersuchung zur Ökologie und zur Rassendifferezierung von *Ips amitinus* und *Ips amitinus* var. montana (Col., Scolytidae). PhD thesis. ETH Zürich, Zürich, 135 pp.

Zúbrik M., Kunca A. & Novotný J. 2008: Hmyz a huby. Národné lesnícke centrum, Zvolen, 178 pp.

Zuk M. 1987: The effects of gregarine parasites, body size, and time of day on spermatophore production and sexual selection in field crickets. Behavioral Ecology and Sociobiology, 21: 65-72.

Zumr V. 1982: The data for the prognosis of spring swarming of main species of bark beetles (Coleoptera, Scolytidae) on spruce (*Picea excelsea* L.). Zeitschrift für Angewandte Entomologie, 93: 305-320.

Zumr V. 1984: Spatial distribution of bark beetles (Coleoptera, Scolytidae) in Norway spruce (*Picea excelsa* Link) and their indifference in relation to forest belts. Lesnictví, 30: 509-523.

Zumr V. 1985: Biologie a ekologie lýkožrouta smrkového (*Ips typographus*) a ochrana proti němu. Academia, Praha, 105 pp.