საქართველოს მეცნიერებათა ეროვნული აკადემიის მოამბე, ტ. 3, №1, 2009
BULLETIN OF THE GEORGIAN NATIONAL ACADEMY OF SCIENCES, vol. 3, no. 1, 2009

Entomology

Occurrence of Pathogens and Nematodes in the Spruce Bark Beetles, *Ips typographus* (Col., Scolytidae) in Borjomi Gorge

Medea Burjanadze, Nana Goginashvili

Vasil Gulisashvili Institute of Forest, Tbilisi

(Presented by Academy Member I. Eliava)

ABSTRACT. Occurrence of pathogens was investigated in the spruce bark beetle *Ips typographus* from three different sampling plots in Borjomi gorge at 950-1100 m above sea level, collected in 2007-2008. Various viral, protozoan pathogen species and nematodes could be observed with a light microscope in the examined beetles. Different collection sites and in different years were observed in the following pathogen complex: Protozoa, *Gregarina typographi,* (7.5% to 49.5%); *Chytridiopsis typographi* (5.8% to 15%), Nosema sp. (5.3%,), virus, like entomopoxvirus - EPV of *Ips typographus* (0.9%) and 3 species of nematodes *Contortylenchus diplogaster, Parasitorhabditis sp., Bursaphelenchus sp.* 40.7-71.4%. Furthermore, pathogens occurred in *Ips typographus* from 3 different sampling plots in an area (one locality) within a distance of a few kilometers. *© 2009 Bull. Georg. Natl. Acad. Sci.*

Key words: *Ips typographus, pathogens, Gregarina typographi, Chytridiopsis typographi, Nosema sp., virus, nematodes, Contortylenchus diplogaster.*

Introduction

The spruce bark beetle, *Ips typographus (Coleoptera: Scolytidae)* is a very important pest insect of Oriental spruce (*Picea orientalis* L) for coniferous forests of Georgia. At present the spruce bark beetle is very active and characterized by a massive increase and formation of focuses in the large tracts of coniferous forests in Georgia. In Borjomi–Bakuriani region the area of conifers forest exceeds 16.677 ha, whereas the damage to spruce stands by this pest 20% in the average. The loss of spruces in some places achieves 70-80% [1]. However, when populations are at high densities the insects can rapidly colonize and kill healthy and vigorous trees [2].

Proceeding from biological particularities it is difficult to develop ways of its control and protect the environment from their invasion. Hence, it is necessary to conduct a survey on the wide-spread population in Georgia in order to reveal new agents among them.

The role of natural enemies (parasitoids, predators, microorganisms) in the dynamics of insect pests is very important. Great attention is paid to their complex study. Bark beetles, settled under bark in one biotope, are assisted by various species of microbes and nematodes. The investigation, identification, selection of bioagents and study of their role in the number of dynamics, serve as a basis for the development of biological protection of conifer forest from *Ips typographus* in the future and assumes one of the important places in integrated pest management (IPM).

The occurrence and epizootiology of pathogens in bark beetles is one of the least studied aspects in their population dynamics. Recent studies produced evidence on several new pathogen species in bark beetles [3-11]. The aim of the present investigations, of identification of bioagents occurring in the populations of Borjomi gorge forest of Georgia, were focused on the complex of

pathogens of bark beetles as a great potential of biological control agents due to their ability to develop strong epizootics that result in natural regulation of *Ips typographus* populations.

Material and Methods

The survey of *Ips typographus* investigation were carried out in spring of 2007- 2008 (in the same month of May) in two different forest district sampling plots - Libani (1100 m a.s.l), Tsagveri (1000 m a.s.l) and Daba (950 m a.s.l) of Borjomi–Bakuriani gorge (800-1300 a.s.λ=41°48′20″– α =43°29′40″).

Adults of bark beetle were collected by cutting infested log sections from spruce trees or by peeling off infested bark or directly taking out of their galleries in the phloem. Log sections and bark with beetles were brought to the laboratory and kept at 15 ±1°C in a refrigerator**.** Beetles were dissected immediately after collection. Only living or slowly moving beetles were removed from their galleries every day and were examined first for macroorganisms, using a stereomicroscope (magnification 8-16-32- 56x). Subsequently, beetles were dissected and whole gut, gonads, fat body and other organs were for examination. Diagnosis and search pathogens were conducted on wet smears, with light microscope (magnification 150-600x) [12]. After fixation with methanol, smears were stained with Giemsa's dye [13] and re-inspected in normal light microscope. The presence of pathogens in different organs or free in the haemolymph was recorded, presence of nematodes was noticed.

Isolation parasitic nematodes will be studied by using generally accepted methods in insect nematology [14,15]. Dissection of beetles in a Petri dish with Ringer's solution under stereomicroscope was carried out by hand anatomy method.

Results

A total of 289 specimens of *Ips typographus* were taken from the inner and outer bark of *Picea orientalis* that were felled and sampled in 2007 and 2008. Date and sites to collecting beetles, mature trees with large, heights and diameters are given in Table 1.

In 2008 in district Daba forest, new colonization tree *Picea orientalis* by *Ips typographus* was discovered. These insects, regarded as secondary bark beetles that feed on subcortical tissue of conifers, are particularly interesting because of their use of aggregation pheromones, which ensures mass colonization of host trees [2].

Reproductive adults bore holes through the outer bark, construct ovipositional galleries within the inner bark and phloem of host trees. Initial entrance tunnels were surrounded by red-to grey-brown boring dust (Fig.1, 2). Average number of galleries with chamber were 4-5 on 1 dcm^2. In each chamber 3 or 4 beetles were observed. Eggs, larvae and pupa have not been observed.

The occurrence of pathogens complex on *Ips typographus* in different years and from different sites of Borjomi-Bakuriani gorge is given in Table 1.

In total, 289 beetles were dissected individually, from three sampling plots. Different microorganisms and nematodes were found in *I.typographus*. The protozoan species, *Gregarina typographi* (Sporozoa, Gregarinidae) described by Fuchs (1915) [16] as a parasite of *Ips typographus* was found in the mid-gut lumen (size of

Table 1.

Ips typographus collected from different plots of the Borjomi gorge (800-1300 a.s.l λ = 41°48′20″ - α =43°29′40″)

#	Date of Collection	Location	Trees, Insects	H* (M)	D* (cm)	P* (year)	Number of collected beetles
1	10.05.07	Libani, district-16	Picea orientalis, *Ips typographus*	32-36	51	90-100	120
2	18.05.07	Tsagveri, district- 9	Picea orientalis, *Ips typographus*	22	28	65-70	56
3	02.05.08	Daba district- 7	Picea orientalis, *Ips typographus*	17-18	35	60-65	113
	Total						289

*** H** – Tree heights; **D** – Tree diameters; **P** – Age of trees

Table 2.

Occurrence of pathogens and nematodes in *Ips typographus* and infection rates (in %) from different sites of Borjomi gorge in 2007-2008 (D.b= Dissected beetles; *G.t= Gregarina typographi*; *Ch.t=Chytridiopsis typographi*; N.sp=Nosema sp.; EPV=entomopox virus; Nem=Nematodes; n=number)

Years	Location	D.b (n)	Infected		*G.t*		*Ch.t*		N.sp		EPV		Nem	
			n	%	n	%	n	%	n	%	n	%	n	%
2007	Libani, district-16	120	66	55	9	7.5	7	5.8	-	-	-	-	51	42.5
2007	Tsagveri, distirct-9	56	44	78.6	7	12.5	-	-	-	-	-	-	40	71.4
2008	Daba, district-7	113	82	72.5	56	49.5	17	15	6	5.3	1	0.9	46	40.7

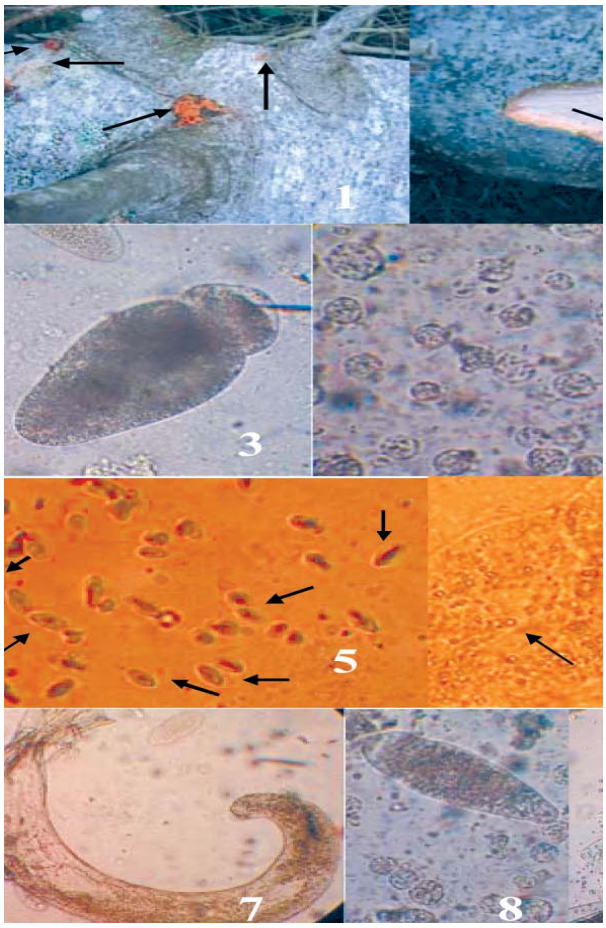

Fig. 1-9. 1 - new colonization tree *Picea orientalis* by *Ips typographus* and holes through the outer bark with boring dust; 2 - galleries and chamber within the inner bark and phloem of host trees; 3 – *Gregarina typographi* (600x); 4- *Chytridiopsis typographi* (300x); 5 – Nosema sp., (600); 6 – Entomopox virus (600x); 7 – *Contortylenchus diplogaster* (150x); mixing infection: 8 - *G. typographi* and *Ch. typographi* ; 9 - *G. typographi* and *Contortylenchus diplogaster.*

gamontes: 50- 75µm x 70-130 µm) of adult beetles from all three locations in Borjomi gorge (Fig.3). Infection rates varied in the beetles from all locations within 7.5%; 12.5 to 49.5%. The pathogens were present in both genders.

Two types of microsporidia are represented in the field population of *I.typographus*: *Chytridiopsis typographi and Nosema* sp. (Fig. 4,5).

In light microscopical observations of fresh smears of *I. typographus*, the Microsporidium *Chytridiopsis typographi* Weiser (1954) [17] could be identified in the cells of the midgut epithelium (Spores 1.5-2.0 x 2.0-2.5 µm; Pansporoblasts (thick walled): 10-20 µm in diameter). This pathogen was found in beetles only at two sites. Infection rates were lower at both sites (5.8% and 15%), but it was absent at the third site.

Nosema sp. could be observed in a few cases in the beetles from the three sites. The pathogens with single binucleate spores in fat body were found. Infection rates were lower - 5.3% (2008).

The virus infection (virus-like entomopox virus - EPV of *Ips typographus)* could be observed only in one dead beetle specimen, collected in Daba sampling plot (2008). The spheroidal inclusion bodies were found in epithelial cells of the gut of the mature beetles (Fig.6). In this case the infection rate was low (0.9%), but it is important that infection was in evidence.

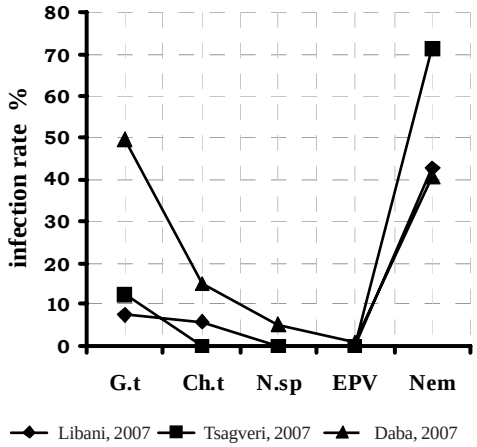

Fig. 10. Comparative number of pathogens occurrence in *Ips typograpus* from different sites of Borjomi gorge in 2007-2008 (G.t= *Gregarina typographi*; Ch.t=*Chytridiopsi typographi*; N.sp=Nosema sp.; EPV=entomopoxvirus; Nem=Nematodes)

Ips typographus were settled under bark in one biotope, assisted by various species of nematodes. As a result of investigation 3 species of nematodes have been revealed: *Contortylenchus diplogaster* (Fig.7), *Parasitorhabditis sp., Bursaphelenchus sp.* They were found in gut, on the surface of body and under elytra of *Ips typographus*, also at the gallery of bark spruce. In 2007 the number of bark beetles infected by nematodes was 42.5-71.4%, in 2008-40.7%.

Bacteria also could be observed but were not identified because of specific difficulties in anatomical association within their host, in isolation and taxonomy.

The comparative number of pathogens occurrence in *Ips typographus* from different sites of Borjomi gorge in different years is given in Fig. 10.

Discussion

The new aspect of this examination is the occurrence of pathogens and nematodes in *Ips typographus* in Georgia. The pathogen complex of the examined *I.typographus* was not very diverse, compared to the results of the study of these bark beetles in European countries [3-8, 11, 18-20].

One of the interesting results was to find numerous pathogen species distributed in *I.typographus* field populations. The prevalence of *Gregarina typographi* was high but showed great variation in number. *G. typographi* was very common in *Ips typographus* populations too and sometimes in high prevalence [6, 11, 21-25]. The pathogen was present in both genders. According to Wegensteiner and Weiser [26].

G. typographi is presumed to appear more often in *I. typographus* males because one male is in contact with two to three females within one breeding system, helping all the females to remove their boring dust and feces, in connection with the external life cycle of *G. typographi*. There is therefore a greater risk for males to get infected in case one or more of the females are infected [24]. However, Gregarines are presumed to be minor virulent pathogens [27], which evidently do not interrupt the migration of beetles.

The natural range of the *Chytridiopsis typographi,* Nosema sp. and virus infection like-entomopox virus rate of infection and number of locality was low.

The pathogens *Chytridiopsis typographi*, Nosema sp. and Entomopox virus, presented here, are the first record of*Ips typographus* in Georgia.

The occurrence of *Ch. typographi* in population *I.typographus* is known from former investigations [6,10,21]. J. Weiser [17] was the first to describe *Chytridiopsis typographi* (formerly *Haplosporidium typographi*) from the gut epithelium of *Ips typographus* [28].

J. Weiser [17] described *Nosema typographi* by means of light microscopy from the adipose tissue of *Ips typograhus* with low infection rates.

The *Ips typographus* Entomopox virus was the first record of a virus disease in the most important European spruce bark beetle species, occurring in the cells of the midgut epithelium [21]. The occurrence of ItEPV in *I.typographus* was reported by different authors [4,20,22,29-32]. Viruses could be very interesting pathogens for microbial control measure, especially from the viewpoint of selectivity and effectiveness. At the moment there is a lack of knowledge.

Nematodes were found free in the haemolymph and in the gut lumen. In the case of presence of female nematodes it was possible to identify *Contortylenchus diplogaster* in the haemolymph. This species was already described for *I. typographus* from Georgia [9,10,13,33]. *Contortylenchus diplogaster* (Tylenchida: Tylenchoidae), is endoparasitic nematode feeding on fat, tissue and haemolymph. The parasite stays for survival in the host for a long time. The gut of bark beetle invised by J3 of this nematode. The species was found in haemolymph and usually one or two, rarely 3-10 exemplars are observed in one bark beetle [16]. *Bursaphelenchus sp.* (Tylenchida: Aphelenchoididae); it is established that this species of nematode parasites under elytra. Under each elytra there were above 50 samples and 70-90% of bark beetle settled by this species. They are facultative ectoparasites. The searching action was observed in the tail part of II stage larvae [33]. *Parasitorhabditis sp.* (Rhabditida: Rhabditidae) was invaded by J2 and J3 stage through the gut of bark beetle. The sex mature forms (♀ & ♂) was found in the gallery of spruce bark. This species was already described in *I. typographus* population from Georgia [33].

The results of our study have underlined the strong need to conduct investigations of bark beetle pathogens in different geographical zones in order to get an accurate knowledge of their diversity and prevalence. Future preservation of bark beetle pathology should establish valuation criteria for assessing the endangering potential of local bark beetle populations. Study of the full spectrum of natural enemies should be considered an environmentally safe means of biological forest protection.

Acknowledgements

This research was supported by the Project GNSF/ST 06/8-112. We thank Prof. G.Kakulia from the Institute of Zoology for confirming the identification of nematodes.

ენტომოლოგია

ბორჯომის ხეობაში გავრცელებული მბეჭდავი ქერქიჭამიას *Ips typographus* (Col., Scolytidae) პათოგენებისა და ნემატოდების გამოვლენა

მ. ბურჯანაძე, ნ. გოგინაშვილი

ვასილ გულისაშვილის სატყეო ინსტიტუტი, თბილისი

(წარმოდგენილია აკადემიის წევრის ი. ელიავას მიერ)

2007-2008 წწ. მბეჭდავი ქერქიჭამია *Ips typographus*-ის პათოგენური მიკროორგანიზმების და პარაზიტული ნემატოდების გამოვლენის მიზნით, ჩატარდა გამოკვლევები და ზრდასრული ხოჭოები შეგროვდა ზღვის დონიდან 950-1100 მ-ზე, ბორჯომის ხეობის სამ სატყეო უბანზე. ხოჭოებში აღინიშნა ვირუსული, პროტოზოული ინფექციები და ნემატოდები. სხვადასხვა სატყეო უბნებზე შეგროვილ საკვლევ მასალში გამოვლინდა შემდეგი პათოგენები: პროტოზოული: *Gregarina typographi* (7.5%–49.5%); *Chytridiopsis typographi* (5.8%–15%), Nosema sp. (5.3%), *Ips typographus*-ის ენტომოპოქსვირუსი – EPV (0.9%) და 3 სახეობის ნემატოდა: *Contortylenchus diplogaster, Parasitorhabditis sp., Bursaphelenchus sp.* (40.7%–71.4%). ის სატყეო უბნები, სადაც შეგროვებული იყო მბეჭდავი ქერქიჭამიას ხოჭოები, ერთმანეთისაგან დაშორებულია რამოდენიმე კილომეტრით.

REFERENCE

1. *B. Tavadze, T. Berozashvili* (2003), Proc. of V. Gulisashvili Institute of Mountain Forestry, vol. **39,** Tbilisi, 169-176.
2. *DL. Wood* (1982), Annu. Rev. Entomol., **42:**179-206.
3. *R. Wegensteiner, U. Händel, E. Fuhrer, J. Weiser* (2000), Proc. of the Intern. Conference. on Forest Ecosystem Restoration. April 10-12, 2000, Vienna, Austria.
4. *B. Haidler, R. Wegensteiner, J. Weiser* (2003), IOBC/wprs Bulletin, **26,** 1: 257-260.
5. *U. Händel, R. Wegensteiner, J. Weiser, Z. Zizka* (2003), J. Pest Science, **76**: 22-32.
6. *U. Händel, R. Wegensteiner* (2005), IOBC/wprs Bulletin, **28,** 3:155-158.
7. *R. Wegensteiner, J. Weiser* (2004), J. Pest Science 77: 221-228.
8. *R. Wegensteiner, R. Kleespies* (2005), IOBC/WPRS Working Group - "Insect Pathogens and Insect Parasitic Nematodes".
9. *M. Burjanadze* (2006), Proc. Georg. Acad. Sci., Biology ser. B, vol.**4,** 4:105-109.
10. *M. Burjanadze, J. Moser, G. Zimmermann, R. Kleespies* (2008), IOBC/wprs Bulletin, **31**: 245-251.
11. *T. Tonka, O. Pultar, J. Weiser* (2007), IOBC/wprs Bulletin, **30 (**1**):** 211-215.
12. *R. Wegensteiner, J. Weiser* (1996), Anz. Schädlingskunde, Pflanzenschutz, Umweltschutz, **69**, 7: 162-167.
13. *J. Weiser* (1977), An Atlas of Insect Diseases. The Hague, Prague, 240 pp.
14. *H. Kaya & P. Stok* (1997), Techniques in Insect Nematology: Manual of Techniques in Insect Pathology (eds. A.Lawrence, L.Lacey), San Diego, 281-324.
15. *E.Pavlovskii* (1957), Metody ruchnogo anatomirovaniya nasekomykh. Moskva, 85 (in Russian).
16. *G. Fuchs* (1915), L.Zool. Jb. Abt. Syst. **38:** 109-222.
17. *J. Weiser* (1954), Vestnik Ceskoslov.zool. spolecnosti, **18:** 217-224.
18. *J. Lipa* (1968), Acta Protozoologica, **6:** 69-77.
19. *K. Purrini* (1978), Anz. Schädlingskunde, Pflanzenschutz, Umweltschutz, **51**: 171-175.
20. *R. Wegensteiner, J. Weiser* (1995), J. Invertebr. Pathol**., 65,** 2: 203-205.

21. *R. Wegensteiner* (1994), IOBC/wprs Bulletin, **17,** 3**:**39-43.
22. *R. Wegensteiner, J. Weiser* (1996), Anz. Schädlingskunde, Pflanzenschutz, Umweltschutz, **69**, 7: 162-167.
23. *R. Wegensteiner* (2004), In: "European bark and wood boring insects in living trees. A synthesis", eds. F. Lieutier, K.Day, A. Battisti, J.C.Grégoire & H. Evans. Kluwer (ISBN 1-4020-2240-9): 291-313.
24. *M. Kereselidze, R. Wegensteiner* (2007), IOBC/wprs Bulletin, **30,** 1**:** 207-210.
25. *D. Takov, D. Doychev, R. Wegensteiner, D. Pilarska* (2007), Acta Zoologica Bulgarica, **59,** 1: 87-96.
26. *R. Wegensteiner, J. Weiser* (2004), J. Pest Science **77**: 221-228.
27. *Y. Tanada & H. Kaya* (1993), Insect Pathology. Acad. Press, San Diego, USA, 666 pp.
28. *J. Weiser* (1970), J. Invertebr. Pathol**. 16:** 436-441.
29. *H. Gasperl* (2002), Diploma Thesis, Universität für Bodenkultur, Wien.
30. *O. Pultar, J. Weiser* (1999), 7th Europ. Meet. IOBC/UFRO, Vienna.
31. *J. Weiser, O. Pultar, Z. Zizka* (2000), UAPPA, Section: **B:**168-172.
32. *Z. Zizka, J. Weiser, R. Wegensteiner* (2001), In: Berger, J. (Ed.): Cells III; Kopp Publ., Česke Budejovice, 214-215.
33. *G. Kakulia* (1989), Paraziticheskie nematody nasekomykh (rabditidy i tilenkhidy) i biologicheskii metod bor'by, Tbilisi 213 (in Russian).

Received December, 2008