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First Record of Entomopoxvirus of *Ips typographus* (Linnaeus) (Coleoptera: Curculionidae, Scolytinae) for Turkey

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Abstract: In present paper, an Entomopoxvirus of *I. typographus* (*It*EPV) in Turkey is reported for first time. *Ips typographus* Entomopoxvirus was found only in Artvin and the total infection rate was 14.7%. It was observed only in the gut epithelium of the host. Spheroids (inclusion bodies) of the virus were rectangular and spherical. The size of rectangular spheroids measured 4 to 10 x 5 to 15 µm and diameter of the spherical ones ranged from 7 to 12 µm. *Ips typographus* Entomopoxvirus infection was also confirmed with a Transmission Electron Microscopy. Spherical to elipsoidal virions in spheroids measured 250 to 280 x 310 to 375 nm in size.

Key words: Biological control, Entomopoxvirus, *Ips typographus*, Turkey

Introduction

European spruce bark beetle *Ips typographus* is an economically important pest in the eastern Black Sea Region of Turkey **(**Eroğlu *et al***.** 2005). Its main host in this region is *Picea orientalis*. Various methods have been used to control this pest, including pheromone traps, chemical pesticides and mechanical control strategies (Toper Kaygin 2007). However, these methods are very expensive and complicated. In addition chemicals control mechanism has harmful effects on the ecosystem, especially natural enemies such as predators and parasitoids. Nowadays environmentalism is getting more popular hence latest studies have focused on using natural enemies in biological control (Wegensteiner 2004, Yaman, Radek 2005, Yaman 2007, Yaman, Radek 2008). Most common biological control agent is microorganisms. Especially entomopathogenic microorganisms are very effective in decreasing density of insect populations (Myers 1988). Despite several studies on the parasites and pathogens of *Ips* spp. from different parts of the world (Wegensteiner, Weiser 1995, Wegensteiner *et al.* 1996, Weiser *et al*. 1998, Weiser *et al*. 2006), there is no record of the pathogens of *I. typographus* in Turkey. In the present study, an entomopoxvirus (*It*EPV) of *I. typographus* is reported for the first time for Turkey.

Materials and Methods

Adult *I. typographus* specimens were collected from spruce *(Picea orientalis)* forests using pheromone traps in Giresun, Rize and Artvin (Turkey), from May to August 2009. Each beetle was dissected in Ringer solution and its intestine was examined microscopically at magnifications of 40x to 1000x. Observed pathogens were measured and photographed using an Olympus BX 51 microscope with a DP-25 digital camera and DP2-BSW Soft Imaging System.

Samples for (TEM) were fixed in 2.5% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4) for 1–2 h, rinsed in cacodylate buffer, post fixed in reduced OsO_4 according to KARNOVSKY (1971) (a

fresh 1:1 mixture of 2% OsO_4 and 3% K_4 {Fe(CN6)} 1.5 h), and rinsed in cacodylate buffer. After dehydration in an increasing ethanol series, the infected beetles were embedded in Spurr's resin (SPURR 1969). Ultra-thin sections were mounted on Pioloform-coated copper grids which were stained with saturated uranyl acetate and Reynold's lead citrate (REYNOLDS 1963). They were examined with TEM microscope.

Results and Discussion

The viral infection was observed in the populations of *I. typographus* in Artvin. During the macroscopic external observations of the infected adults there were no any visible symptoms confirming viral infection. Under light microscope, high number of inclusion bodies (IBs) formed by the virus was observed in the adult host. Viral inclusion bodies showed the typical characters of entomopoxvirus (EPV) recorded in bark beetles (Wegensteiner, Weiser 1995). The viral infection was observed only in the gut epithelium and was not present in other tissues (Fig. 1 A, B). Wegensteiner, Weiser (1995) recorded that EPV in *I. typographus*, infecting the host midgut epithelium only, differs from all other EPVs. Viral inclusion bodies called spheroids were rectangular and hemispherical in shape (Fig. 1-B, 2). *It*EPV infection was also confirmed with TEM. Spherical and elipsoidal virions in spheroids measured 250 to 280 x 310 to 375 nm in size (Fig. 2 A-D).

Measurement of size of virions in spheroid in Händel *et al*. (2003), Wegensteiner, Weiser (1995) and our study is different (Table1). It is seen that there are some morphological differences among Turkish and European isolates. It is possible that two different virus strains may occur in Turkey and in Europe. Murillo *et al*. (2001) suggest that some strains of nucleopolyhedrovirus isolated from different geographies may present better insecticidal activities which make them more suitable for their host control and show important differences in biological activity. Similar judgment is possible for EPV isolates. Additionally, Asia is a potential source of new and interesting virus strains (Murillo *et al*. 2001).

*It*EPV was found in adult beetles collected only from Artvin. Sixty nine of the 468 investigated beetles were infected with *It*EPV during three months (May, June and July). The total rate of *It*EPV infection was 14.7% during the three months. Till now, *It*EPV was found only in *I. typographus* and *I. amitinus* (Wegensteiner, Weiser 1995, Takov *et al*. 2006,

Table 1. The *It*EPV infections in *Ips* spp.

Virus	Host	Percent of infected individuals (%)	Size of spheroids		Year	Locality	Literature
			spherical	Rectangular (µm)			
*It*EPV	*Ips typographus*	0.3 1.1	5 to 12	4 to 10 x 5 to 11	1993	Austria	Wegensteiner and Weiser, 1995
*It*EPV	*Ips typographus*	≤0.5 min 18.1 max	-	-	1997-1999	Austria	Händel et al., 2003
*It*EPV	*Ips amitinus*	0.3	-	3-14 x5-14	1999	Austria	Händel et al., 2003
*It*EPV	*Ips typographus*	2.3 2.6 9.8 0.9		-	2003-2005	Bulgaria	Takov et al., 2006
*It*EPV	*Ips typographus*	0.9		-	2007-2008	Georgia	Burjanadze and Goginashvili, 2009
*It*EPV	*Ips typographus*	4.8		-	1995	Germany	Wegensteiner and Weiser, 1996
*It*EPV	*Ips typographus*	7.9		-	2003	Bulgaria	Takov et al., 2007
*It*EPV	*Ips typographus*	14.7	7 to 12	4 to 10 µm x 5 to 15	2009	Turkey	Present study

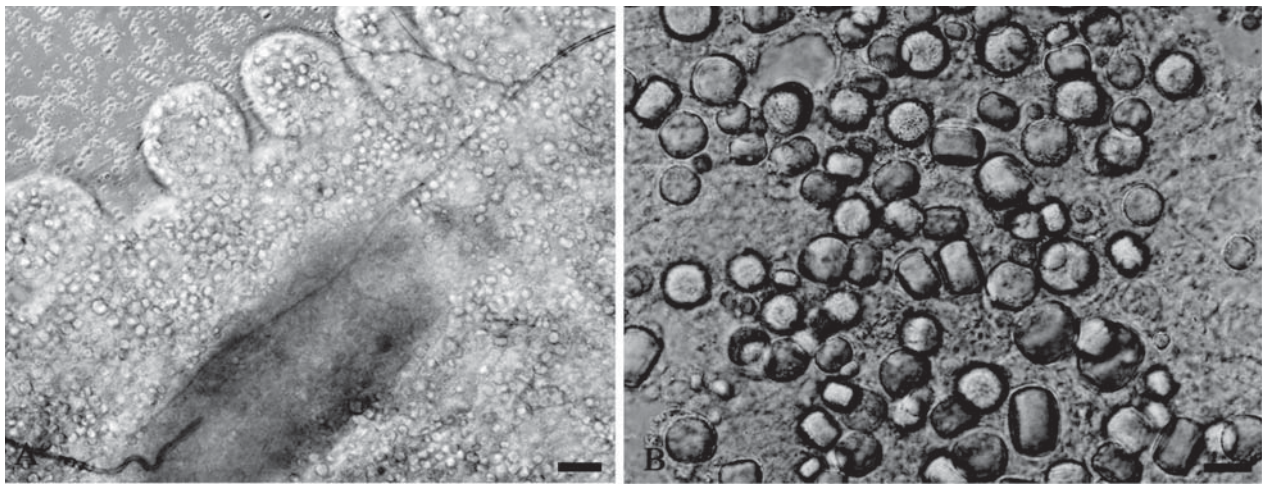

Fig. 1. Spheroids of *It*EPV from *I. typographus* under light microscope (Bars: A: 20µm, B: 100 µm).

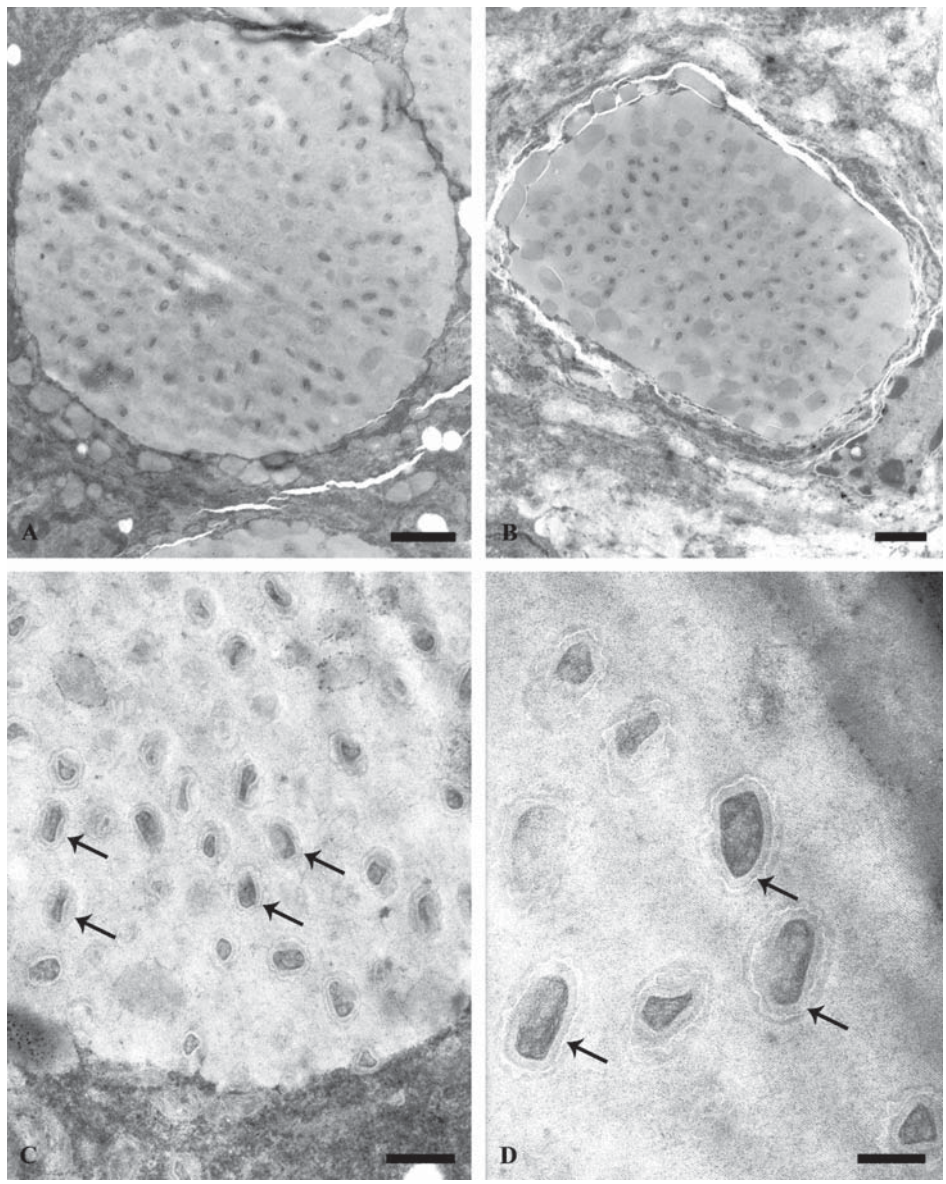

Fig. 2. Spheroids of *It*EPV from *I. typographus* under TEM Microscope (Bars: A: 1000 nm, B: 1000 nm, C: 400 nm, D: 200 nm).

Takov *et al*. 2007, Burjanadze, Goginashvili 2009).

According to the results mentioned above the infection rates were higher in Turkey, Germany and Bulgaria, but lower in Georgia and Austria. Georgia, Turkey and Bulgaria are neighbor countries but the reported infection rates of *It*EPV were different. To explain these differences more extensive studies are needed in this area.

Till now, there is no any virus record from *I. typographus* in Turkey although Asia is a potential source of new and interesting virus strains (Murillo *et al*. 2001). *It*EPV presented here is the first pathogen of *I. typographus* for Turkey and could be an alternative biological control agent of this insect, because EPV gives very promising results in biological control of insects. Recently, TONKA *et al*. (2009) accomplished to test the laboratory management of entomopoxvirus in *Ips typograhus*. They found that 595 of the tested 1142 beetles were infected by the entomopoxvirus. The authors were able to keep this virus in the laboratory for several weeks.

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