Silva Balcanica, 11(1)/2010

PROTOZOAN PARASITES AND PATHOGENS OF FOREST PEST ARTHROPODS

Vassil Golemansky[1], *Daniela Pilarska*[1], *Georgi Georgiev*[2], *Danail Takov*[1], *Milcho Todorov*[1], *Plamen Pilarski*[3]
[1]Institute of Zoology, [2]Forest Research Institute, [3]Institute of Plant Physiology
Bulgarian Academy of Sciences – Sofia

Abstract

Study of protozoan parasites and pathogens of forest pest insects is a perspective concerning the biological control. The subject of our investigations in this respect were 2 arthropod species which are serious pests in the forests, nurseries, greenhouses, etc.: *Lymantria dispar* and *Oxidus gracillis*. Our research on the pathogens of *L. dispar* started since 1995 in cooperation with European and USA investigators. As a result of our study three protozoan parasites – *Vairimorpha disparis*, *Nosema lymantriae* and *Endoreticulatus schubergi* (Microsporidia) were observed on this pest. In 2000 experimental studies on the introduction of an entomopathogenic fungus *Entomophaga maimaiga* into *L. dispar* populations in Bulgaria have started. The results obtained showed that the fungus has been successfully introduced in Bulgaria and practical results are expected in the next years. *O. gracilis* is a new invasive species for Bulgarian fauna from Far East which lives so far in greenhouses, nurseries, etc. Its wider distribution in the country is expected at our climatic conditions. Three protozoan parasites were recorded in this millipede – *Stenophora nematoides*, *Stenophora robusta* and *Fonsecaia polymorpha* (Apicomplexa: Sporozoa). While *S. nematoides* and *S. robusta* showed a high prevalence, *F. polymorpha* was detected in a few individuals only.

Key words: *Lymantria dispar, Oxidus gracilis, Entomophaga maimaiga,* microsporidia, gregarines

Study of protozoan parasites and pathogens of forest pest insects is a perspective concerning the biological control. The subject of our investigations were two arthropod species which are serious pests in the forests, nurseries, greenhouses, etc.: *Lymantria dispar* Linnaeus (Insecta, Lepidoptera) and *Oxidus gracilis* (C.L. Koch), (Myriapoda, Diplopoda).

Lymantria dispar

The gypsy moth *L. dispar* is a pest naturally spread in the Palearctic zoogeographical region – Europe, Asia, Japan and North Africa. It periodically causes outbreaks and, in years of high population densities, defoliations are regularly observed in Bulgarian oak forests (Georgiev et al., 2007). The attacked trees weaken. They can be affected by other stress factors and may die after a single defoliation in a subsequent year.

Currently, pest management organizations in South and Central European countries use the bacterial pathogen *Bacillus thuringiensis* Berliner var. *kurstaki* (*Btk*) and a broad spectrum of insecticides such as Dimilin and Mimic to manage damaging gypsy moth larval populations. However their use and particularly that of chemical pesticides, is controversial because they are not highly specific and may affect many

other non-target insect species and aquatic organisms, and thus have a negative effect on the forest ecosystems biodiversity (Miller, 1990). The use of pathogens such as protozoa, microsporidia and fungi is a preferred alternative.

Microsporidia are intracellular parasites that usually cause chronic disease in their insect hosts. Deleterious effects include reduced egg hatch, increased larval mortality, growth delays, and lower fecundity in their insect hosts. Several species of microsporidia occur in the forest defoliator *L. dispar* and are considered to be important components of the natural enemy complex in Central and Eastern Europe.

Our research on the parasitic protozoa and pathogens of *L. dispar* started since 1995 in cooperation with USDA Forest Service, University of Illinois – USA, University of Applied Sciences, Eberswalde – Germany, BOKU – Austria, Charles University – Czech Republic.

As a result of our studies, three species of microsporidia have been recovered from Bulgarian populations of the gypsy moth *L. dispar* in a cooperation including European and USA investigators (Pilarska et al., 1998; Maddox et al., 1999; Solter et al., 2000; Hylis et al., 2006; Vavra et al., 2006, etc.). The life history, morphology, host tissue specificity, virulence and persistence, and biology of these microsporidian isolates have been intensively studied to elucidate interactions with the host and to facilitate decisions regarding their use in biological control programs (Linde et al., 2000; Solter at al., 2000, 2005; Goertz et al. 2004, 2007; Pilarska et al., 2006b).

Vairimorpha disparis Vavra, 2007 was recorded for first time in Bulgaria in 1984 in *L. dispar* population from Rupite (South Bulgaria). It is transmitted horizontally in the host population. Vertical transmission from infected females to their offspring has not been recorded (Goertz, Hoch, 2008). The prevalence of *Vairimorpha disparis* in Rupite *L. dispar* population during the period 1984-1997 varied from 1.8% in 1997 to 30% in 1984. There was no correlation between the prevalence of microsporidium and host density but the microsporidium persisted in the population even when *L. dispar* densities were low during the 13-year period (Pilarska et al., 1998).

Nosema lymantriae Weiser, 1957 was recorded for first time in 1996 repeatedly found in 1997 *L. dispar* population from Levishte and Veslets (North Bulgaria) and in 2009 in Stryama and Gorni Domlyan (Central Bulgaria). It infects the fat body, silk glands, Malpighian tubules, and gonads, but the silk glands appear to be the primary target tissue. This species is transmitted both horizontally, e.g. by contaminated silk trails, and vertically through the eggs (Goertz, Hoch, 2008). *Nosema lymantriae* spores are binucleate and typical for the genus. Its prevalence depends of population density and was not found when the population has collapsed (Pilarska et al., 1998).

Endoreticulatus schubergi (Zwolfer) was firstly recorded in 1996 and repeatedly found in 1997, 1998 in Central Bulgaria in *L. dispar* population from Asenovgrad. This species infects the midgut cells and is only horizontally transmitted; spores are vomited and are present in the faeces (Goertz, Hoch, 2008). The prevalence of infection increased with the age of *L. dispar* larvae (Pilarska et al., 1998). It was found that this microsporidium has a little influence on growth and development of the host (Hoch

et al., 2009; Kereselidze et al., 2010). The investigations on the biology of the three microsporidian species continue and study on developing methods for their release into *L. dispar* populations are developing.

The fungus *Entomophaga maimaiga* Humber, Shimauzu & Soper (Entomophtorales: Entomophtoraceae) was isolated and described as a pathogen on *L. dispar* in Japan. It was introduced into North America (Massachusetts) in the beginning of XX century (Speare, Colley, 1912). In 1985-1986 a second introduction of the fungus was conducted in New York and Virginia states (Nielsen et al., 2005). Recently *E. maimaiga* have been recorded in 17 states in the USA (Hoover, 2000; Balser, Baumgand, 2001; Hajek et al., 2005) and in Ontario, Canada (Howse, Scarr, 2002).

Bulgaria is the first country in Europe where *E. maimaiga* was successfully introduced and became established (Pilarska et al., 2000, 2006a, 2007; Georgiev et al., 2007). The pathogen was imported from the USA and introduced in seven different *L. dispar* populations in the region of State Forestries (SF) Svoge (1996 and 2001), Karlovo (1999), Assenovgrad (2001), Stryama (2005), Nova Zagora (2008), Popovo (2009) and Gorna Oryahovitsa (2009). *E. maimaiga* was not recovered after the first introduction, however after the second in the region of SF Karlovo it was found that 6.3% of the collected larvae were infected (Pilarska et al., 2000). During the period 2000-2003, approximately 11.0% of the host larvae collected in the region of SF Karlovo and SF Svoge were infected with the fungus (Pilarska et al., 2006a).

In 2005, an epizooty caused by *E. maimaiga* occurred at four different sites in Bulgaria (SF Haskovo, Kirkovo, Botevgrad and Govezhda) located 30-70 km from the introduction sites in 2000 and 2001 (Georgiev et al., 2007; Pilarska et al., 2007). In 2008 and 2009 the range of the fungus was expanded by its introduction into three new sites (SF Nova Zagora, SF Popovo and SF Gorna Oryahovitsa). Moreover in 2009 *E. maimaiga* was recovered in two more localities – SF Ravna gora and Zvezdets. We believe that *E. maimaiga* could be a preferred alternative to the use of both microbial and chemical pesticides, which are expensive to apply and toxic to various non-target organisms. Furthermore the establishment of *E. maimaiga* all over Bulgaria will reduce the use of insecticides and will decrease the frequency of *L. dispar* outbreaks.

Oxidus gracilis

The flat-backed millipede *O. gracilis* (= *Orthomorpha gracilis* C.L. Koch) (Diplopoda: Paradoxosomatidae) is an introduced alien invasive species in Bulgaria (Stoev, 2004). It originates from South Eastern Asia, but so far it is registered from many American and European countries, inhabiting mainly the humid warm habitats, the tropical section of Botanic gardens, green and hot houses in many temperate regions. The millipede often develops in massive populations in the invaded habitats and is considered as a serious pest in the gardens. So far, *O. gracilis* was observed in Bulgaria in some hot houses only, but we suppose that it is distributed in other habitats too, because of its well known high adaptive potential.

Data for presence of some eugregarines in *O. gracilis* were published by many authors (Ellis, 1912; Pinto, 1922; Hukui, 1952; Geus, 1969; Lipa, 1967, etc.). So far,

7 species of eugregarines were observed and described as new taxa from *O. gracilis: Stenophora robusta* Ellis, *S. ozakii* Hukui, *S. orthomorphae* Lipa, *S. poznanienzis* Lipa, *S. orthomorphae* Geus, *S. vermiformis* Geus and *Fonsecaia polymorpha* Pinto, but some of them are probably synonyms. No other protozoan pathogens were recorded from *O. gracilis* up to now.

The object of our investigations were living specimens of *O. gracilis*, collected from 3 green hot houses in Sofia: Botanic garden of 'St Kliment Ohridski' University of Sofia, Botanic garden of Bulgarian Academy of Sciences and Glasshouse of University of Forestry – Sofia (Darvenitza). A total of 122 specimens collected during the period 1996-2010 were investigated.

As a result of our study 3 species of eugregarines were observed in the gastrointestinal tract of the examined diplopods:

Stenophora nematoides Leger et Duboscq. It is the most common intestinal parasite of the specimens from the three examined populations, found in 74 animals of different age (total prevalence 59%). *S. nematoides* was firstly described by Leger, Duboscq (1903) from the diplopode *Enthosolenium italicum* (=*Stosatea italicum*) and later recorded by Hukui (1952) in *O. gracilis* too. Young cephalonts were observed in the epithelial cells of the intestine, and free sporonts and gamonts were often observed in the intestinal tube.

S. robusta Ellis. The total prevalence of the examined diplopods was relatively high too – 46%. Only sporonts and mature gamonts were observed in the middle part of the intestinal tube. The species was recorded in other diplopods in Europe also (Geus, 1969).

Fonsecaia polymorpha Pinto. This eugregarine was quite rare in our observation. So far it is considered as monospecific for *O. gracilis.* It was observed in 6 examined animals only (total prevalence 4%).

So far, a limited number of protozoan parasites were found in *O. gracilis* and that is probably due to the fact that the flat-backed millipede is a recently introduced alien species in Europe and in Bulgaria. We expect that *O. gracilis* will be soon a real constant component of the natural habitats in Bulgaria and it must be controlled as a potential pest in our country also.

Acknowledgements: We are especially indebted to the National Science Fund of Bulgaria grant DO-02-191/2008 and DO-02-282/2008 for their contribution to this work. Part of the results were presented at the International Scientific Conference 'Forestry: Bridge to the Future', Sofia, 13-15 May 2010.

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