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PATHOGENS AND PARASITOIDS OF FOREST PEST INSECTS IN THE REGION OF THE FOREST PROTECTION STATION PLOVDIV (BULGARIA), DURING THE PERIOD 1990 - 2017

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Abstract

During the period 1990-2017, a survey of the entomopathogens and parasitoids of several pest insects was conducted, including the lepidopterans *Lymantria dispar, Euproctis chrysorrhoea, Leucoma salicis, Malacosoma neustria, Orthosia cerasi, Aporia crataegi, Operophtera brumata, Eilema complana, Tortix viridana, Archips xylosteana, Paranthrene tabaniformis*, *Gypsonoma aceriana*, *Thaumetopoea pityocampa, T. solitaria*, *Phyllocnistis unipunctella*, the coleopterans *Saperda populnea, Ips typographus, I. acuminatus, I. sexdentatus, Pityogenes chalcographus*, *Dryocoetes autographus*, *Hylurgops palliatus*, *Phyllobius* sp. and the hymenopterans *Diprion pini*, *Neodiprion sertifer*, *Gilpinia* sp. and *Tremex fuscicornis*. As a result of these studies 5 viruses, 1 protozoan species, 7 microsporidian species, 5 species of entomopathogenic fungi and 46 parasitoid species have been documented in 27 host insects collected in the region of the Forest Protection Station Plovdiv. The first successful introduction in Bulgaria of the entomopathogenic fungus *Entomophaga maimaiga* in populations of *L. dispar* was conducted in 1999 in the region of the Forest Protection Station at Plovdiv (in the village of Gorni Domlyan, Karlovo Forestry) and 7 subsequent introductions were later performed in the region of the station. As a result, *L. dispar* density has been maintained at low levels in that area and only 60 hectares were sprayed with insecticides for *L. dispar* control in the last 18 years. Another success was the first field release in Europe and

Bulgaria of the entomopathogenic fungus *Entomophaga aulicae* in a healthy population of the brown tail moth, *Euproctis chrysorrhoea*, in 2016 in the village of Zhenda (Kardzhali Forestry). Investigations in 2017 showed that 19% of *E. chrysorrhoea* larvae sampled from release sites had died due to infection by *E. aulicae*.

Key words: Forest Protection Station – Plovdiv, insect pests, pathogens, parasitoids, *Entomophaga maimaiga*, biological control

Forest pest insects are a major cause of economic and aesthetic loss in forestry systems and are of particular concern as forest habitats become more fragmented, land use pressures increase, and climate change impacts forest ecosystems.

Many insects in Bulgaria attained pest status when plantation forestry increased in importance and created monocultures that favour pest outbreaks. The most significant pests of Bulgarian forests are species in the orders Coleoptera and Lepidoptera. In the coniferous forests bark beetles (Coleoptera: Curculionidae) and the processionary moth, *Thaumetopoea pityocampa* (Denis & Schiffermüller) (Lepidoptera: Notodontidae) are of greatest concern. Important pests of broadleaf forests include moths in the families Erebidae [*Lymantria dispar* (Linnaeus), *Euproctis chrysorrhoea* (Linnaeus), *Leucoma salicis* (Linnaeus)], Tortricidae [*Archips xylosteana* (Linnaeus), *Tortrix viridana* Linnaeus] and Geometridae (*Operophtera brumata* (Linnaeus), *Erannis defoliaria* (Clerck), *Alsophila aescularia* (Denis & Schiffermüller), *Alsophila aceraria* Denis & Schiffermüller). These pests can cause significant damage and each year huge areas of forests are affected. Forestry administrations are committed to reducing the use of pesticides in the environment and to emphasizing the use of biological control agents.

Pathogens and parasitoids as natural enemies are such control agents that play important roles in regulating population densities of forest pest insects.

In the region of Plovdiv Forest Protection Station the main pests during the last twenty years are the lepidopterans *T. pityocampa*, *L. dispar*, *E. chrysorrhoea*, *A. xylosteana*, *T. viridana*, *E. defoliaria*, *O. brumata* and the bark beetles *Ips typographus* (Linnaeus), *Ips acuminatus* (Gyllenhal), *Ips sexdentatus* (Börner) and *Pityogenes chalcographus* (Linnaeus).

In order to set the stage for practical application of conservation practices and control measures for the biological protection of our forest ecosystems, a survey of entomopathogens and parasitoids of several pest insects was conducted in the region of the Forest Protection Station Plovdiv.

In this paper we present results of investigations on pathogens and parasitoids of pest lepidopterans, coleopterans and hymenopterans.

As a result of our studies 5 viruses, 1 protozoan species, 7 microsporidian species, 5 species of entomopathogenic fungi, and 46 parasitoid species have been documented in 27 insect species collected in the region of the Forest Protection Station Plovdiv during the years 1990 – 2017.

Viruses

Five viruses were detected in the lepidopterans *L. dispar*, *T. pityocampa*, *L. salicis*, the bark beetle *I. typographus* and the hymenopteran *Neodiprion sertifer* (Geoffroy).

The first virus was the *L. dispar* nuclear polyhedrosis virus (*Ld*NPV; Baculoviridae). *Ld*NPV infects all tissues and develops in the nuclei of infected cells. It was detected in 1999 in 2.3% of the gypsy moth larvae collected in Gorni Domlyan village, Karlovo State Forestry, where in the same year the first successful introduction of the fungus *Entomophaga maimaiga* Humber, Shimazu & Soper (Entomophthoromycota, Entomophthorales) was conducted (Pilarska et al., 2000). The same virus was detected in 2003 in 85.2% in larvae collected in the region of Chekeritsa State Game Enterprise (Annual Report of Forest Protection Station Plovdiv, 2003) .

In 2004, after an outbreak of the satin moth, *L. salicis*, in the region of Plovdiv State Forestry, an epizootic caused by an undetermined nuclear polyhedrosis virus was observed. As a result, 69% of the collected larvae died with symptoms of NPV (Annual Report of Forest Protection Station Plovdiv, 2004).

In 2008, a granulosis virus, *Tp*GV (Baculoviridae), was detected in dead larvae of the pine processionary moth, *T. pityocampa*, collected from the village of Banya, Karlovo State Forestry. Viral infections caused 100% mortality of pine processionary moth larvae (Annual Report of Forest Protection Station Plovdiv, 2008).

During the period 2003 and 2011, an entomopox virus, *It*EPV Wegensteiner & Weiser (Entomopoxviridae) was observed in spruce bark beetles, *I. typographus*, collected in Beglika, Batak State Forestry and Borino, Borino State Forestry. *It*EPV spheroids were detected in midgut epithelial cells. Prevalence of this virus in the beetles collected in Beglika in 2005 was 16.6 and the virus caused 4.7% infection in 2011. In Borino the virus was detected in 2003 in 3.8% of the investigated individuals (Takov et al., 2006, 2007).

The fifth virus documented in the region of Forest Protection Station Plovdiv was the nuclear polyhedrosis virus of the satin moth *Neodiprion sertifer* (Geoffroy). It was detected in the region of Karlovo State Forestry in 2016, where 1.8 ha of pine trees were infested with the satin moth. This virus caused 50% mortality of the sawfly larvae (Annual Report of Forest Protection Station Plovdiv, 2016).

Protozoa

Protozoa of the genus *Gregarina* (Apicomplexa, Eugregarinoida) were detected in three bark beetle species: *I. typographus*, *I. acuminatus* and *I. sexdentatus*. *Gregarina typographi* Fuchs was detected in *I. typographus* collected in three sites: Beglika, Borino and Yundola from 2003 to 2011 (Takov et al., 2006, 2007, 2012). This gregarine was observed in the midgut lumen of beetles collected in Beglika in 2005 and 2011, with a prevalence of 16.6% and 31.7%, respectively. In Borino, 7.3% of all investigated beetles were infected with *G. typographi* in 2003 and 5.9% in 2005. The pathogen was also found in 5.1% of individuals collected in

Yundola in 2009. *Gregarina* spp. were also found in *I. acuminatus* (prevalence of 7.2% in Borino in 2005) (Takov et al., 2007) and in *I. sexdentatus* (prevalence of 11.1% in Slaveyno State Forestry in 2017) (Annual Report of Forest Protection Station Plovdiv, 2017).

Microsporidia

In total, seven microsporidian species (Microsporidia) were detected in the gypsy moth *L. dispar*, *E. chrysorrhoea*, tortricids and *I. typographus.*

The first microsporidian documented in the study period was *Endoreticulatus schubergi* (Zwolfer) Cali & Garhy in *L. dispar* in 1996 and 1997, near Asenovgrad (Asenovgrad State Forestry). It infects the gut epithelium and is transmitted horizontally by oral uptake of spores. The prevalence of *E. schubergi* in the host population increased with the growth of the host larvae as the season progressed. The prevalence of *E. schubergi* in 1996 varied from 2.0 to 11.5% (average 6.1%) and in 1997 varied from 27.0 to 56.0% (average 3.2%) (Pilarska et al., 1998).

In the same host, a second microsporidian species, *Nosema lymantriae* Weiser was detected in two sites (Chekeritsa State Game Enterprise and Gorni Domlyan, Karlovo State Forestry) in 2009 and 2010. This microsporidium infects the fat body, silk glands, Malpighian tubules and gonads. It is transmitted horizontally and transovarially. The prevalence in Chekeritsa was 13.9% (2009) and 1.9% in Gorni Domlyan (2010) (D.P., unpublished).

In 2000, 72 brown tail moth, *E. chrysorrhoea*, populations in Balkan, Sredna Gora, Sakar and Rodope Mountains were screened for microsporidian infections. In 13 populations collected in Sakar and Rodope Mountains, a *Nosema* sp. was detected, and in 8 populations in Balkan, Sredna Gora and Sakar Mountains an *Endoreticulatus* sp. was observed (Pilarska et al., 2001a). The prevalence of *Nosema* sp. was 37.9% (Rodope Mt.) and 21.4% (Sakar Mt.), and the prevalence of *Endoreticulatus* sp. in Balkan and Sredna Gora Mountains was 11.1% and 10.0%, respectively (Annual Report of Forest Protection Station Plovdiv, 2000).

An undetermined *Nosema* isolate was discovered in the fat body tissues and silk glands of larvae of *Orthosia cerasi* (Fabricius) from Gorni Domlyan in 2010, with a prevalence of 20.0% (Pilarska et al., 2017).

In 2011, during an investigation of newly hatched tortricid larvae from the region of Rakitovo and Alabak Forestry Stations, another *Nosema* sp. was detected. Its prevalence was 12.5% in the larvae from Rakitovo and 5.0% in the larvae from Alabak (Annual Report of Forest Protection Station Plovdiv, 2011).

Chytridiopsis typographi Weiser was found in the bark beetle *I. typographus*, collected in 2003 in Borino and in 2011 in Beglika. Spores were observed in the cells of the midgut epithelium, enclosed in sporophorous vesicles or without such enclosures. Prevalence was 1-6% in beetles from Beglika and 3.8% in beetles from Borino (Takov et al., 2006, 2012).

Fungi

In total, five species of entomopathogenic fungi were detected in the lepidopterans *L. salicis*, *E. chrysorrhoea*, *L. dispar*, *E. defoliaria*, *Thaumetopoea solitaria* (Freyer), *T. pityocampa*, *Malacosoma neustria* (Linnaeus), *Aporia crataegi* (Linnaeus), *T. viridana*, in the bark beetles *Dryocoetes autographus* (Ratzeburg), *Hylurgops palliatus* (Gyllenhal), and in the weevil *Phyllobius* sp.

The first fungal entomopathogen discovered was *Entomophaga aulicae* (E. Reichardt) Humber (Entomophthoromycotina, Entomophthorales) in 16 outbreak populations of brown tail moth, *E. chrysorrhoea*, in Balkan, Sakar, Sredna Gora and Rodope Mountains in 2000. The fungus was identified as the cause of high larval mortality with the result of a reduced population density of this moth (Pilarska et al., 2001b).

In 2016, mortality of brown tail moth larvae was also observed in the region of Asenovgrad State Forestry. Microscopic analyses showed that mortality was also caused by *E. aulicae.* In order to preserve inoculum of the fungus, dead brown tail moth larvae were collected and stored in the soil humus layer (Pilarska et al., 2016a).

The third fungal pathogen identified was the hypocrealean fungus *Beauveria bassiana* (Balsamo) Vuillemin (Ascomycota, Hypocreales),found in dead larvae of *L. salicis* (Markova, Georgiev, 1992), *T. solitaria* (Mirchev et al., 2012), *L. dispar, A. crataegi, E. defoliaria, T. pityocampa*, *M. neustria, T. viridana, D. autographus, H. palliatus,* and *Phyllobius* sp., collected from 20 sites (Draganova et al., 2013).

Aspergillus niger van Tieghem (Ascomycota, Eurotiales) was found in *E. defoliaria,* another *Aspergillus* sp. was detected in *T. pityocampa, L. dispar* and *T. viridana,* and in *T. pityocampa, M. neustria* and *T. viridana* an entomopathogenic fungus belonging to the genus *Fusarium* Link was discovered (Ascomycota, Hypocreales) (Draganova et al., 2013).

Introduction and release of entomopathogenic fungi

The first successful introduction of the entomopathogenic fungus *Entomophaga maimaiga* into populations of *L. dispar* in Bulgaria was conducted in 1999 in the region of the Forest Protection Station at Plovdiv, in the village of Gorni Domlyan (Karlovo Forestry) (Pilarska et al., 2000). In the period 2000-2004 the prevalence of *E. maimaiga* in local populations of *L. dispar* ranged from 6.1% (2000) to 15.9% (2004). However, the fungus persisted in the introduction site and in 2005, massive epizootics caused by this fungus were recorded in State Forestry Haskovo and State Forestry Kirkovo (Pilarska et al., 2007). In 2012 fungal epizootics were reported from the regions of State Forestry Haskovo and Harmanli with larval mortality of 100%. In the period 2005-2013, seven subsequent introductions were performed in the region of the Forest Protection Station Plovdiv (Georgiev et al., 2013; Pilarska et al., 2016b). As a result, the *L. dispar* density has remained at low levels in that area and only 60 hectares had to be sprayed with insecticides for *L. dispar* control during the last 18 years.

The release of the entomopathogenic fungus *Entomophaga aulicae* using the methodology for introduction of *E. maimaiga* was an attempt to conduct biological control against one of the most important insect pests – the brown tail moth. Therefore, in 2016, we conducted the first

controlled field release of *E. aulicae* in Bulgaria, in a healthy population of *E. chrysorrhoea* in the village of Zhenda (Kardzhali State Forestry) (Pilarska et al., 2017b). The release of *E. aulicae* was performed in the beginning of November 2016 using the inoculum from an outbreak site of *E. chrysorrhoea* in Asenovgrad. Our investigations in 2017 showed that the introduction was successful, with 19% of *E. chrysorrhoea* larvae sampled from the release sites dying due to infections caused by *E. aulicae* (Annual Report of Forest Protection Station Plovdiv, 2017).

Parasitoids

In total, 46 parasitoid species were detected in 11 insect hosts: the small poplar longhorn beetle *Saperda populnea* (Linnaeus), the lepidopterans *Phyllocnistis unipunctella* (Stephens) (syn. *Phyllocnistis suffusella* Z.), *Gypsonoma aceriana* (Duponchel), *Paranthrene tabaniformis* (Rottemburg), *L. salicis*, *T. pityocampa*, *T. solitaria*, the sawflies *Diprion pini* (Linnaeus), *N. sertifer*, *Gilpinia* sp., and the tremex wasp *Tremex fuscicornis* (Fabricius).

In *S. populnea* larvae, 5 hymenopteran and dipteran parasitoids were found in Plovdiv, Ognyanovo, Simeonovgrad, Manole, and Klisura regions: *Iphiaulax impostor* (Scopoli) (Hymenoptera: Braconidae), *Dolichomitus populneus* (Ratzeburg), *Xylophrurus lancifer* (Gravenhorst), *Gelis ornatulus* (Thomson) (Hymenoptera: Ichneumonidae) and *Billaea irrorata* (Meigen) (Diptera: Tachinidae) (Georgiev, 2001a; Georgiev et al., 2004). *G. ornatulus* was found as a hyperparasitoid.

In *P. unipunctella* larvae, 10 primary and secondary hymenopteran parasitoids were found in the Pazardzhik region: *Chrysocharis nephereus* (Walker), *Chrysocharis* sp. 1, *Chrysocharis* sp. 2, *Cirrospilus elegantissimus* Westwood, *Cirrospilus lyncus* Walker, *Cirrospilus pictus* (Nees), *Kratoisma usticrus* (Erdös), *Sympiesis sericeicornis* (Nees), *Sympiesis* sp. and *Tetrastichus* sp. (Hymenoptera: Eulophidae) (Georgiev, Pelov, 1996; Georgiev, 2005). The hyperparasitoid *Tetrastichus* sp. was detected in *K. usticrus*, *C. nephereus*, *S. gyorfii*, *S. sericeicornis* and *Sympiesis* sp. The host mortality caused by all parasitoid species reached up 77.6%, and *K. usticrus* had the strongest impact on the host population (Georgiev, 2005).

In *G. aceriana* larvae, 6 hymenopteran parasitoids were found in the Pazardzhik region: *Bracon variator* Nees, *Macrocentrus marginator* (Nees), *Orgilus obscurator* (Nees), *Apanteles erevanicus* Tobias (Hymenoptera: Braconidae), *Pristomerus rufiabdominalis* Ucida and *Pristomerus vulnerator* (Panzer) (Hymenoptera: Ichneumonidae) (Georgiev, 1995). The total mortality of *G. aceriana* larvae caused by parasitoids ranged between 5.9 and 28.6% in the first host generation and between 35.8-44.4% in the second host generation (Georgiev, Samuelian, 1999). *A. erevanicus* was the most common parasitoid, causing host mortality of up to 27.5%.

In larvae of *P. tabaniformis*, 5 parasitoids were found in the Pazardzhik region: *Apanteles evonymellae* (Bouché), *Bracon intercessor* Nees (Hymenoptera: Braconidae), *Eriborus terebrans* (Gravenhorst), *Pristomerus vulnerator* (Panzer) and *Dolichomitus populneus* (Ratzeburg) (Hymenoptera: Ichneumonidae) (Georgiev, Tsankov, 1995; Georgiev et al., 2001). Host mortality caused by all parasitoid species was 27.6%, and *A. evonymellae* was the most common parasitoid, causing host mortality of 18.6%.

In *L. salicis* larvae and pupae, 9 parasitoid species were found in the Pazardhik region: *Theronia atalantae* (Poda) (Hymenoptera: Ichneumonidae), *Brachymeria intermedia* (Nees), *Brachymeria minuta* (Linnaeus) (Hymenoptera: Chalcididae), *Monodontomerus aereus* Walker (Hymenoptera: Torymidae), *Compsilura concinnata* (Meigen), *Exorista larvarum* (Linnaeus), *Pales pavida* (Meigen) (Diptera: Tachinidae), *Agria mamillata* (Pandelle) (Diptera: Sarcophagidae) and *Megaselia* sp. (Diptera: Phoridae) (Georgiev, 1996a). Six species (*B. intermedia, M. aereus, C. concinnata, E. larvarum, P. pavida* and *A. mamillata*) were confirmed as primary parasitoids. One species (*T. atalantae*) was both a primary and secondary parasitoid, and two species (*B. minuta* and *Megaselia* sp.) as hyperparasitoids on *P. mamillata*. The parasitoids reduced the pest population by 36.7%, and *A. mamillata* and *B. intermedia* had the strongest impacts.

In *T. pityocampa,* 7 egg parasitoids were found in Kurtovo, Banya, Yanino, Kardzhali, Ivaylovgrad, Rozino, and Klisura regions: *Baryscapus servadeii* (Domenichini), *Baryscapus transversalis* Graham, *Pediobius bruchicida* Rondani (Hymenoptera: Eulophidae), *Anastatus bifasciatus* (Geoffroy), *Eupelmus vesicularis* (Retzius) (Hymenoptera: Eupelmidae), *Ooencyrtus pitiocampae* Mercet (Hymenoptera: Encyrtidae) and *Trichogramma embryophagum* Hartig (Hymenoptera: Trichogrammatidae) (Tsankov et al., 1996a, 1996b; Mirchev et al, 1998; Mirchev, Tsankov, 2001; Mirchev, 2005; Mirchev et al., 2011). Six of these species are primary parasitoids, and one (*B. transversalis*) is a hyperparasitoid of *B. servadeii* and *O. pitiocampae*.

Four species were found as egg parasitoids of *T. solitaria* in the regions of Ivaylovgrad and Madzharovo for the first time: *Anastatus bifasciatus* (Hymenoptea: Eupelmidae), *Ooencyrtus pityocampae* Mercet, *Ooencyrtus masii* (Mercet) and *Ooencyrtus* sp. (close to *O. indefinitus* Myartseva) (Hymenoptea: Encyrtidae) (Mirchev et al., 2014; Boyadzhiev et al., 2017).

In *D. pini* pupae in Tpopovgrad region, 4 hymenopteran and dipteran parasitoid species were found: *Agrothereutes adustus* (Gravenhorst), *Exenterus amictorius* (Panzer), *Olesicampe* sp. (Hymenoptera: Ichneumonidae) and *Drino inconspicua* (Meigen) (Diptera: Tachinidae) (Georgiev, Bochev, 1996). Parasitoids caused mortality rates of 31.6% in *D. pini* and *A. adustus* had the strongest impact on the host population

In Ognyanovo region, 3 parasitoids of *N. sertifer* were detected. Two species, *Dipriocampe diprioni* (Ferriére) (Hymenoptera: Tetracampidae) and *Baryscapus oophagus* (Otten) (Hymenoptera: Eulophidae), were reared from host eggs (Georgiev, 2001b), and *Agrothereutes adustus* from host pupae (Georgiev, Kolarov, 1999). *D. diprioni* was found as a primary parasitoid, and *B. oophagus* as a hyperparasitoid.

Two parasitoids were detected and reported for first time in Bulgaria: *Diplostichus janithrix* (Hartig) (Diptera: Tachinidae) in *Gilpinia* sp. in the Ognyanovo region (Georgiev, 1996b), and *Ibalia leucospoides* (Holchenwarth) (Hymenoptera: Ibaliidae) in *Tremex fuscicornis* in the Manole region (Ljubomirov et al., 2006).

We conclude that the pathogens and parasitoids found in the region of the Forest Protection Station Plovdiv form an extensive natural enemy complex, maintaining the population densities of insect pest species at lower levels. This is particularly important for species that potentially

increase to outbreak densities and whose populations then cause economic losses in forest ecosystems or severe allergies to humans and animals (*L. dispar*, *E. chrysorrhoea*, *E. defoliaria*, *T. pityocampa*, *L. salicis*, *T. solitaria*, etc.). Some fungal pathogens (*E. maimaiga*, *E. aulicae*, *B. bassiana*) are already used in forest protection as classical or augmentation biological agents.

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