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LABORATORY OBSERVATIONS ON LARVAL HATCHING OF *THAUMETOPOEA PITYOCAMPA* EARLY DEVELOPING PHENOLOGICAL FORM IN EASTERN RHODOPES, BULGARIA

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Abstract

The hatching dynamics of larvae of early developing phenological form of pine processionary moth (*Thaumetopoea pityocampa*) based on 93 egg batches was monitored under laboratory conditions. The egg batches were collected on July 12, 2018 in a 30-year-old *Pinus nigra* plantation, at an altitude of 450 m in the region of Fotinovo village, Kirkovo State Forestry, Eastern Rhodopes. Newly hatched larvae started appearing at the beginning of July 2018, and continued for a month, until August 8, 2018. The peak lasted for 13 days, from the 19th to 31th of July, during which 80.6 % of the larvae hatched. Field observations conducted in parallel, showed that the larval development in the studied habitat lasted for about three months.

Key words: larval development, larval phenology, pine processionary moth.

Introduction

Pine processionary moth, *Thaumetopoea pityocampa* (Denis & Schiffermüller, 1775) (Lepidoptera: Notodontidae) is a Mediterranean species which is one of the most dangerous pests in these latitudes. The species was subject of many studies regarding its geographic expansion, in response to the increasing winter temperature and other climate anomalies (Battisti et al. 2005, 2006; Toffolo et al. 2006; Robinet et al. 2007; Mirchev et al. 2011a). In this regard, observations of the seasonal phenology within its natural range are very important to understand pest's response to such climatic patterns (Bellin et al. 1990; Schmidt 1989, 1990; Battisti et al. 2015; Roques et al. 2015; Mirchev et al. 2017). The phenology of *T. pityocampa* varies with climatic conditions (Démolin 1969; Robinet et al. 2015). Adult emergence, immediately followed by mating and egg laying, occurs between June (in colder mountainous areas) and October (in areas with Mediterranean climate) (Aimi et al. 2006). A unique population of summer phenological form was discovered in 1997 in Leiria, Portugal (Pimentel et al. 2006), where it continues to be present at high densities. In the same stands and trees, the summer form coexists with the typical winter one. Studies have been conducted and hypotheses put forward

about the variations in the phenology of the species and the emergence of new phenological forms (Santos et al. 2007, 2011, 2013; Battisti et al. 2015; Burban et al. 2016).

T. pityocampa was found for first time in Bulgaria in 1906 (Drenovsky 1923). The biology of the species was initially observed in the region of Belovo, located between Rila, the Rhodopes and Sredna Gora Mountains (Buresch 1915). It was established that adult moths start to emerge in June, and new-hatched larvae can be found at the end of August. Russkoff (1929–1930) reported that adults emerged from the end of June to late August, with the highest intensity in July, based on observations in the region of Velingrad, Rakitovo and Asenovgrad (Western Rhodope Mountains). According to the author, the time of pine processionary moth development does not always coincide with data published in the entomological literature. He pointed out that in several regions of the country, two phenological forms occurred, with identical morphological aspect. For example, in the region of Devin, Velingrad (Western Rhodopes) and Karlovo (Central South Bulgaria), the larvae usually descend for hibernation in the soil, unlike Kostenets (Rila) and Chepelare (Western Rhodopes) where the larvae spend winter in the nests.

Zankov (1960) studied *T. pityocampa* biological features across many regions in Bulgaria and highlighted the variations in its phenology. In the regions of Razlog, Blagoevgrad and Sandanski (Southwestern Bulgaria) it was noticed that larvae of the pine processionary moth hibernated in the nests. The period of pupation was quite extended, the first larvae descending to the soil at the end of April, and the last ones during the first part of June. In Velingrad, Hissarya and Karlovo the larvae hatched at the end of July or the beginning of August. In the middle of October, approximately 40 % of larvae in single nests descended for hibernation in the soil, and the remaining until the second half of February. Tsankov et al. (1996) reported that in the region of Banya near Karlovo, pine processionary moth development started a month earlier, by comparison to the rest of the country. Studies carried out in Bulgaria significantly contributed to clarify the distribution of both forms, the early developing form, and the typical Mediterranean, or winter (late developing) one (Zankov 1960; Tsankov et al. 1996; Mirchev et al. 2011b, 2016, 2017, 2019).

Recently, the early developing phenological form of *T. pityocampa* was observed in pine stands of Kirkovo State Forestry in Eastern Rhodopes (Mirchev et al. 2019). However, the larval hatching period and dynamics of this form have not been previously studied, neither in Kirkovo nor in other localities in Bulgaria.

This note presents the results of a laboratory study on larval hatching of the early developing phenological form of *T. pityocampa* in a new habitat in Bulgaria.

Materials and Methods

Ninety-three egg batches of *T. pityocampa* were collected on July 12, 2018 from a 30-year old plantation of *Pinus nigra* Arn. in the region of Fotinovo village (Kirkovo State Forestry, Eastern Rhodopes) (Fig. 1). The site is located at an altitude of 450 m AMSL, coordinates 41º22’37.5’’ N, 25º19’18.5’’ E.

After collection, egg batches were transported to the laboratory of Forest Research Institute in Sofia. Each egg batch was put in a test tube covered with cotton

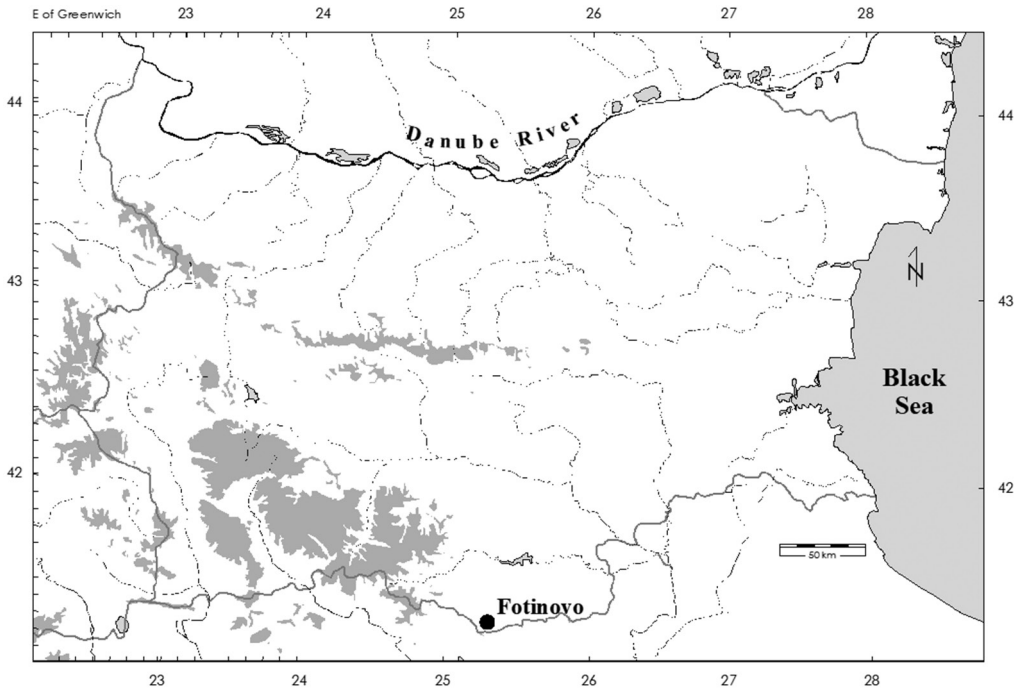

Fig. 1. Studied locality of *T. pityocampa* in Bulgaria.

stopper, and kept under laboratory conditions at 20–22 °C. The samples were observed daily for the presence of larval hatching.

Additionally, in the studied site, the contents of 20 nests of *T. pityocampa* were investigated on the 25th of September and 9th of October 2018, in order to determine the larval instars and the time of descending for pupation in the soil.

Results

The population density of *T. pityocampa* was high in the pine stands studied, with over 10 egg batches on a single tree (observation 12.07.2018). During the collection of biological material, larval hatching was recorded in five egg batches (5.4 %). The neonate larvae were found feeding at the base of the needles, near the batches (Fig. 2). It was assumed that the larvae were 3–4 days old, i.e. hatching occurred around 7th–10th of July 2018.

Under laboratory conditions, the period of larval hatching lasted for one month – from 7th of July to 8th of August 2018 (Fig. 3). Mass hatching was observed for 13 days (July 19–31), when the percentage of newly-hatched larvae attained 80.6 %. In this period, daily hatching ranged from 2.2 % to 16.1 %. This rate was significantly higher and over 6 times larger than at the beginning and end of the period.

The analysis of 20 nests on 25th September 2018 showed that pine processionary moth larval population was in 4th–5th instar, with 5th instar larvae dominating. The observation carried out on 9th of October 2018 showed that, in two of

Fig. 2. Neonate *T. pityocampa* larvae emerging from an egg batch.

20 studied nests, the larvae had already descended into the soil for pupation, and in the remaining nests all larvae were in 5th instar. Thus, the larval development of early developing phenological form lasted for about three months.

Discussion

The region studied is located at 40 km from the Aegean Sea, but the influence of the sea is obstructed by Gyumyurdzhinski Snezhnik ridge in the Eastern Rhodope Mountains. The region is characterized by relatively mild winters with average temperatures above 0 °C, and hot, dry summers. The period with temperatures above 32 °C (critical for the development of pine processionary moth eggs and young larvae) lasts only for 10–12 days. In the region of Sandanski, the habitat of the winter phenological form of *T. pityocampa*,

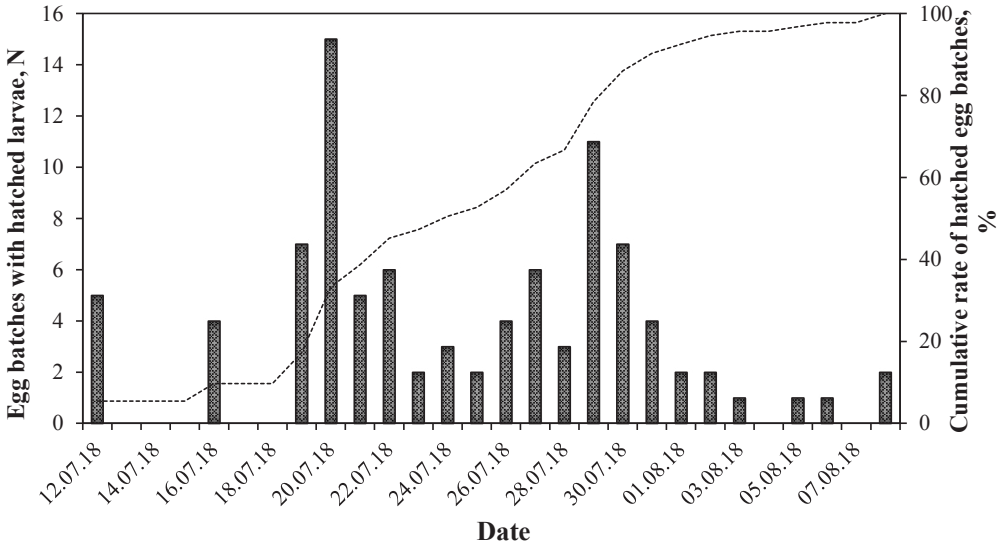

Fig. 3. Hatching period in the laboratory.

Note: daily number of egg batches showing the first hatched larvae (columns); cumulative percentage of egg batches with hatched larvae (dotted line).

that period lasts on average 20 days, although in some years, like between 2012 and 2015, it varied between 38 and 43 days (Mirchev et al. 2018).

Several authors published data on the phenology in various regions of its natural range. Thus, in areas under typical Mediterranean climate, adult emergence can be delayed until September (Battisti et al. 2015). For example, in Greece (Kassandra peninsula and Kalogria), larval hatching was observed from the end of September to the end of October (Schmidt 1989, Bellin et al. 1990). In most of the other areas (colder sites at high elevation, or high latitude), the flight period can last from late June – early July to late August – early September, with a flight peak in July (Roques et al. 2015). In the atypical phenological form in Bulgaria, hatching was observed from early July to early August. In the summer form in Portugal, egg laying lasts from the end of April to the beginning of July, and hatching starts a month later, between the end of May and the beginning of July (Battisti et al. 2015).

Variations in the phenology of *T. pityocampa* have been explained by habitats' climatic conditions. Battisti et al. (2015) reported that egg hatching in colder sites in Greece occurred in July and in warmer sites in October. A similar interpretation was proposed for the closely related species *T. wilkinsoni* Tams in Turkey, where egg hatching occurred in August at colder sites, and in November in warmer ones (Battisti et al. 2015). Yet, these assertions were not completely confirmed by the data obtained in Bulgaria. In the coldest habitats, corresponding to the Northwestern zones of *T. pityocampa* range expansion, only the winter form phenology of the species was observed (Mirchev et al. 2011b, 2016).

Santos et al. (2011) pointed that the summer its form found in Portugal, should be regarded as a separate phenological population. According to Santos et al. (2007), the case under consideration refers to a mode of sympatric speciation in which the differentiation of populations is primarily due to a phenological shift without habitat or host shift. Comparing the summer phenological form of *T. pityocampa* with the winter one, Santos et al. (2013) found the presence of phenotype differentiation: variations in the fertility of female moths, in the shape and colour of the covering scales of egg batches, as well as in the species composition and the impact of the egg parasitoid complex on *T. pityocampa* population density. Both monitoring of adult flight patterns and genetic analyses demonstrated that these two forms are strongly isolated from each other (even where they are sympatric) (Burban et al. 2016). Since the Portuguese summer population already differs significantly in its genetics, morphology, ecology and physiology, it should not be directly compared with all other phenological forms of *T. pityocampa* (Godefroid et al. 2016).

In conclusion, the results of the present survey expand the knowledge on larval hatching and development of the established atypical (early developing) form of pine processionary moth in Bulgaria. The phenological changes in this economically important insect pest can affect both its management and public health in attacked forest areas.

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References

Aimi A., Zocca A., Minerbi S., Hellrigl K., Gatto P., Battisti A. 2006. The outbreak of the pine processionary moth in Venosta/Vinschgau: ecological and economic aspects. Forest Observer 2/3: 69–80.

Battisti A., Stastny M., Netherer S., Robinet C., Schopf A., Roques A., Larsson S. 2005. Expansion of geographic range in the pine processionary moth caused by increased winter temperatures. Ecological Applications 15(6): 2084–2096.

Battisti A., Stastny M., Netherer S., Robinet C., Schopf A., Roques A. 2006. A rapid altitudinal range expansion in the pine processionary moth produced by the 2003 climatic anomaly. Global Change Biology 12: 662–671.

Battisti A., Avci M., Avtzis D.N., Ben Jamaa M.L., Berardi L., Berretima W., Branco M., Chakali G., El Alaoui El Fels M.A., Frérot B., Hódar J.A., Ionescu-Măläncuş I., İpekdal K., Larsson S., Manole T., Mendel Z., Meurisse N., Mirchev P., Nemer N., Paiva M.-R., Pino J., Protasov A., Rahim N., Rousselet J., Santos H., Sauvard D., Schopf A., Simonato M., Yart A., Zamoum M. 2015. Natural History of the Processionary Moths (*Thaumetopoea* spp.): New Insights in Relation to Climate Change. In: Roques A. (Ed.) Processionary Moths and Climate Change: An Update. Springer: 15–79.

Bellin S., Schmidt G.H., Douma-Petridou E. 1990. Structure, ooparasitoids spectrum and rate of parasitism of egg-batches of *Thaumetopoea pityocampa* (Den. & Schiff.) (Lep., Thaumetopoeidae) in Greece. Journal of Applied Entomology 110: 113–120.

Burban C., Gautier M., Leblois R., Landes J., Santos H., Paiva M.-R., Branco M., Kerdelhué C. 2016. Evidence for low-level hybridization between two allochronic populations of the pine processionary moth, *Thaumetopoea pityocampa* (Lepidoptera: Notodontidae). Biological Journal of the Linnean Society 119: 311–328.

Buresch I. 1915. Die Nachtschmetterlinge Bulgariens mit besonderer Berücksichtigung der schädlichen Arten. Travaux de la Société Bulgare des sciences naturelles, Bd VII: 9–100 (in Bulgarian, German summary).

Démolin G. 1969. Bioecología de la procesionaria del pino *Thaumetopoea pityocampa* Schiff. Incidencia de los factores climáticos. Boletín del Servicio de Plagas Forestales 12: 9–24.

Drenovsky A. 1923. A harmful species of pine caterpillar in Bulgaria. Forestry review 9: 234–247 (in Bulgarian).

Godefroid M., Rocha S., Santos H., Paiva M.-R., Burban C., Kerdelhue C., Branco M., Rasplus J.-Y., Rossi J.-P. 2016. Climate constrains range expansion of an allochronic population of the pine processionary moth. Diversity and Distributions: 1–13. Available at: https://doi.org/10.1111/ddi.12494.

Mirchev P., Georgiev G., Matova M. 2011a. Prerequisites for expansion of pine processionary moth *Thaumetopoea pityocampa* (Den. & Schiff.) in Bulgaria. Journal of Balkan Ecology 14(2): 117–130.

Mirchev P., Georgiev G., Tashev A. 2011b. Instar structure of caterpillars of pine processionary moth in Bulgaria during the cold period of the year. Forest Science, Sofia, 1–2: 37–46 (in Bulgarian, English summary).

Mirchev P., Georgiev G., Georgieva M., Bocheva L. 2016. Impact of low temperatures on pine processionary moth (*Thaumetopoea pityocampa*) larval survival in Bulgaria. Silva Balcanica 17(1): 51–58.

Mirchev P., Georgiev G., Georgieva M., Matova M., Zaemdzhikova G. 2017. Enlargement of the pine processionary moth (*Thaumetopoea pityocampa*) range in Bulgaria. Forest Review, Skopje 48(1): 4–7.

Mirchev P., Georgiev G., Tsankov G., Georgieva M., Petkov P., Zaemdzhikova G., Topalov P., Hlebarska S., Matova M., Kitanova S., Go-

lemanski V., Pilarska D., Todorov M., Takov D., Pilarski P., Hubenov Z., Boyadzhiev P., Bocheva L., Barta M. 2018. Report, first stage of the project of National Scientific Fund DN 01/17, 22.12.2016 Expansion of pine processionary moth (*Thaumetopoea pityocampa* (Denis & Schiffermüller, 1775) (Lepidoptera, Thaumetopoeidae) in Bulgaria – a dangerous allergen and economical important pest in the pine ecosystems. 205 p.

Mirchev P., Georgieva M., Zaemdzhikova G., Matova M., Hlebarska S., Filipova E., Georgiev G. 2019. Phenological form diversity of *Thaumetopoea pityocampa* in Bulgaria. Topola/Poplar 203: 65–69.

Pimentel C., Calvão T., Santos M., Ferreira C., Neves M., Nilsson J-A. 2006. Establishment and expansion of a *Thaumetopoea pityocampa* (Den. & Schiff.) (Lep. Notodontidae) population with a shifted life cycle in a production pine forest, Central-Coastal Portugal. Forest Ecology and Management 233: 108–115.

Roques A., Rousselet J., Avci M., Avtzis D.N., Basso A., Battisti A., Ben Jamaa M.L., Bensidi A., Berardi L., Berretima W., Branco M., Chakali G., Çota E., Dautbašić M., Delb H., El Alaoui El Fels M.A., El Mercht S., El Mokhefi M., Forster B., Garcia J., Georgiev G., Glavendekić M.M., Goussard F., Halbig P., Henke L., Hernańdez R., Hódar J.A., İpekdal K., Jurc M., Klimetzek D., Laparie M., Larsson S., Mateus E., Matošević D., Meier F., Mendel Z., Meurisse N., Mihajlović L., Mirchev P., Nasceski S., Nussbaumer C., Paiva M.-R., Papazova I., Pino J., Podlesnik J., Poirot J., Protasov A., Rahim N., Peña G.S., Santos H., Sauvard D., Schopf A., Simonato M., Tsankov G., Wagenhoff E., Yart A., Zamora R., Zamoum M., Robinet C. 2015. Climate Warming and Past and Present Distribution of the Processionary Moths (*Thaumetopoea* spp.) in Europe, Asia Minor and North Africa. In: Roques A. (Ed.). Processionary Moths and Climate Change: An Update. Springer: 81–161.

Robinet C., Baier P., Pennerstorfer J., Schopf A., Roques A. 2007. Modelling the effects of climate change on the potential feeding activity of *Thaumetopoea pityocampa* (Den. & Schiff.) (Lep., Notodontidae) in France. Global Ecology and Biogeography 16(4): 460–471.

Robinet C., Laparie M., Rousselet J. 2015. Looking beyond the large scale effects of global change: local phenologies can result in critical heterogeneity in the Pine Processionary Moth. Frontiers in Physiology 6: 334. DOI: 10.3389/fphys.2015.00334.

Russkoff M. 1929–1930. Beitrag zum Studium der Biologie und Ökologie des Pinenprozesspinners (*Thaumetopoea pityocampa* Schiff.) in Bulgarien. Sonderabdruck aus dem Jahrbuch der Univertät in Sofia, Land- und Forstwirtschaftliche Fakultät, Bd. VIII: 261–284 (in Bulgarian, German summary).

Santos H., Rousselet J., Magnoux E., Paiva M.-R., Branco M., Kerdelhue C. 2007. Genetic isolation through time: allochronic differentiation of a phenologically atypical population of the pine processionary moth. Proceedings of the Royal Society B 274: 935–941.

Santos H., Burban C., Rousselet J., Rossi J.-P., Branco M., Kerdelhue C. 2011. Incipient allochronic speciation in the pine processionary moth (*Thaumetopoea pityocampa*, Lepidoptera, Notodontidae). Journal of Evolutionary Biology 24(1): 146–158.

Santos H., Paiva M.-R., Rocha S., Kerdelhue C., Branco M. 2013. Phenotypic divergence in reproductive traits of a moth population experiencing a phenological shift. Ecology and Evolution 3(15): 5098–5108.

Schmidt G.H. 1989. Life cycles of *Thaumetopoea* species distributed in different regions of Europa, North Africa and Near East. In: Schmidt G.H. (Ed.) Thaumetopoea-Symposium 5–7 July 1989 at the University of Hannover. Proceeding 'On the biology and control of *Thaumetopoea* spp.': 24–30.

Schmidt G.H. 1990. The egg-batches of *Thaumetopoea pityocampa* (Den. & Schiff.) (Lep., Thaumetopoeidae): Structure, hatching of the larvae and parasitism in southern Greece. Journal of Applied Entomology 110: 217–228.

Toffolo E., Bernardinelli I., Stergulc F., Battisti A. 2006. Climate change and expan-

sion of the pine processionary moth, *Thaumetopoea pityocampa*, in Northern Italy. IUFRO Working Party 7.03.10 Proceedings of the Workshop 2006, Gmunden, Austria: 331–340.

Tsankov G., Schmidt G.H., Mirchev P. 1996. Structure and parasitism of egg-batches of a processionary moth population different from *Thaumetopoea pityocampa* (Den. & Schiff.) (Lep. Thaumetopoeidae) found in Bulgaria. Bollettino di Zoologia agraria e di Bachicoltura, Ser. II 28(2): 195–207.

Zankov G. 1960. Untersuchungen über einige merkmale aus der Biologie und Oekologie Kiefernprozessionsspinners (*Thaumetopoea pityocampa* Schiff.) bei uns in Verbindung mit den Methoden zu seiner Bekämpfung. Ministerium für Land und Forstwirtschaft, Forschungsinstitut für Forst und Forstwirtschaft, Wissenschaftliche Arbeiten, Bd. VIII: 231–262 (in Bulgarian, German summary).