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The Hatching Period of Winter and Summer Populations of Thaumetopoea pityocampa (Lepidoptera: Notodontidae) in Bulgaria

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Abstract. The dynamics of the larval emergence of the "summer form" and "winter form" of *Thaumetopoea pityocampa* was studied in the period July-October 2020 in laboratory conditions. The biological material was collected from two sampling sites – Sandanski in the western foothills of Prirn Mts. and Dobrostan on the northern slope of the Rhodopes Mts. The region of Sandanski is occupied by the winter form and the region of Dobrostan – by the summer form. The phenological calendars of the two populations of *T. pityocampa* were found to be in agreement with the known temperature thresholds of the species. The culmination of the larvae hatching of the winter form was in early October – well after midsummer heats, while the culmination of the summer forms was in early August and coincided with the hottest period. In both populations, however, the culmination of hatching occurred at average monthly maximum air temperatures of about 28°C.

Key words: larvae hatching, phenological forms, pine processionary moth, temperature threshold.

Introduction

The pine processory moth (PPM) *Thaumetopoea pityocampa* (Denis & Schiffermüller, 1775) (Lepidoptera: Notodontidae) is a forest insect, which extends its geographical range as a result of climate warming (IPCC, 2007; Raev et al., 2011). The climate change affects the vital activity of insects through various processes, which may increase their presence in some areas and decrease it in others. For example, rising winter temperatures are expected to favor the more northern outbreaks of southern pine beetles, but to reduce them in more southern ones (Ungerer et al., 1999). A similar shift of the attacks of the pine processionary moth was observed in Bulgaria. Zaemdzhikova et al. (2020a) reported an increase in the frequency of *T. pityocampa* attacks at the northern border of its range and a reduction in the south. In any case, the global warming, at least initially, will benefit thermophilic insects, increasing the frequency of pest outbreaks. The expansion of the PPM in Europe is due to rising winter temperatures, which favor the winter feeding and survival of caterpillars in new habitats located to the north and higher altitudes (Battisti et al., 2005, 2006; Buffo et al., 2007; Robinet et al., 2007, 2015).

Expansion of PPM has also been established in Bulgaria (Mirchev et al., 2017; Zaemdzhikova et al., 2018).

Phenology is the time schedule of seasonal activities of plants and animals such as flowering or reproduction (Moore & Allard, 2008). As the phenology of organisms depends on temperature, it can be expected to be affected by climate change. Expected reactions to rising temperatures are faster life cycles of insects, earlier emergence of their larvae and adults and increasing duration of the flight period (Menéndez, 2007). Members of the order Lepidoptera again provide the best examples of such phenological changes (Roy & Sparks, 2000; Stefanescu et al., 2003).

There are two phenological forms of PPM – "summer form" (SF) and "winter formr" (WF), as they are often termed in the entomological literature (Mirchev et al., 2019). It is not yet known whether there are genetic differences between them or they are behavioral strategies of the same species in response to different environments. For this reason, many authors prefer the terms summer population and winter population. Whatever the truth, two types of phenological calendars of the species are observed. The summer form flies early (in May-June, before the summer solstice) (Zaemdzhikova, 2020), its larvae feed in summer, pupate in the soil in autumn and overwinter there (Georgieva et al., 2019; Mirchev et al., 2019; Santos et al., 2011, 2013). The winter form flies late (in July-August, after the solstice), its larvae develop in winter, pupate in the soil in the spring and remain there in the obligatory diapuse until July (Mirchev et al., 2017; Santos et al., 2007, 2011, 2013; Tsankov & Mirchev, 2000; Zaemdzhikova, 2020).

The air temperature is a major limiting factor for the vital activity of insects. Especially the temperature extremes can be fatal for the survival of the PPM. However, the temperature thresholds are different for the different instars so the temperature should affect the two forms differently. For example, for the survival of WF caterpillars in the period October-February, temperatures at -13°C (L2) and -16°C (L3/L4) are lethal (Buffo et al., 2007), as well as some diurnal temperature fluctuations - the insufficient number of the so-called "favorable days for feeding" (the days with an activation temperature >9°C during the day and air temperatures >0°C at night) is also deadly (Battisti et al., 2015). These temperature thresholds are essential for the survival of WF, but do not affect SF. Conversely, summer extremes (air temperatures above 32°C and 40°C) affect rarely the development of the WF, but are crucial for the survival of young caterpillars of SF, which develope during the hot months (Georgieva et al., 2019; Robinet et al., 2013; Santos et al., 2011).

The winter form is worldwide much more widespread and long known (Drenovski, 1923; Mirchev et al., 2000, 2019; Zankov, 1960). However, Bulgaria is perhaps unique, at least for now, in that the summer form is also widespread (about the half of the studied sites) and long known. In 1926, for the first time, Chorbadzhiev reported it – he reported caterpillars collected from the Rhodope Mts., which completed their development in the autumn. Later, in the 1950s, Zankov (1960) also reported habitats in the Sub-Balkan Valleys and the Central Rhodopes, where some of the caterpillars completed their development before the cold winter months. These are perhaps the first scientific reports of atypical PPM populations, which were re-discovered in Portugal in 1997 much later (Pimentel et al., 2006).

The aim of the present work is to compare the periods of larval emergence of the phenologically different populations of the PPM and to look for a correlation with the temperature thresholds of the species.

Material and Methods

The biological material (egg-batches) of PPM was collected from two sampling sites – the lands of Dobrostan vill. and the town of Sandanski, for which according to literature data the phenological form of the species is known: SF in Dobrostan and WF – in Sandanski (Mirchev et al., 2019; Tsankov & Mirchev, 2000). Dobrostan is located in a mountainous region on the northern periphery of the Western Rhodopes Mts., in South-central Bulgaria (41°54′05.57′′N, 24°55′22.84′′E, 1110 m a.s.l.). Sandanski is located in the Sandanski-Petrich valley, at the foot of Pirin Mts., in the Southwestern Bulgaria (41°34′07.53′′N, 23°17′33.84′′E, 310 m a.s.l.). Both sites are within "Southern-Frontier Forest Vegetation Province", which has a transitional-Mediterranean climate (Sabev & Stanev, 1959).

The egg-batches of *T. pityocampa* were collected in Austrian black pine plantations (*Pinus nigra* Arn.) in July-September 2020, i. e. before the caterpillars start hatching in both sites. The eggs were stored in laboratory at room temperature (daytime 22°C, nighttime 17°C). The number of hatching egg-batches was counted daily.

To characterize the climate data, we used the average monthly maximum air temperatures in Sandanski and Dobrostan provided by the National Institute of Meteorology and Hydrology – NIMH (Bulgaria).

Results

The dynamics of the hatching by sites is shown in Table 1. The hatching of the summer form began at the end of July, that of winter form – in mid-September, i.e. a month and a half later. The average duration of the hatching period of the both populations was about 3 weeks, slightly more in winter populations than in summer ones.

Figures 1 and 2 show the timing and dynamics of larval hatching in both sites. To describe the daily dynamics of hatching, both the curve of the empirical distribution and the cumulative distribution are given. From empirical distributions it is seen that in both forms the distribution was multimodal, i. e. there was more than one culmination and more precisely – three of them. In both figures two weaker culminations precede the peak of caterpillar hatching.

Another criterion of the culmination of hatching was the 50th percentile read from the cumulative distribution. The peak of summer form's hatching was observed in the first ten days of August (Fig. 1) and that of the winter form – in early October (Fig. 2). This result shows, that the culmination of hatching in the winter populations in Sandanski occurred a month and a half later than that of the early developing summer population in Dobrostan.

Table 1. Periods of hatching of collected egg-batches of the PPM's summer and winter form in laboratory.

Observations	Dobrostan (SF)	Sandanski (WF)
Date of collection of egg batches	18.08.2019	14.08.2019
Egg batches, number	46	25
Beginning of hatching	26.07.2020	16.09.2020
50% of hatching	07.08.2020	02.10.2020
End of hatching	14.08.2020	11.10.2020
Duration (days)	19	25

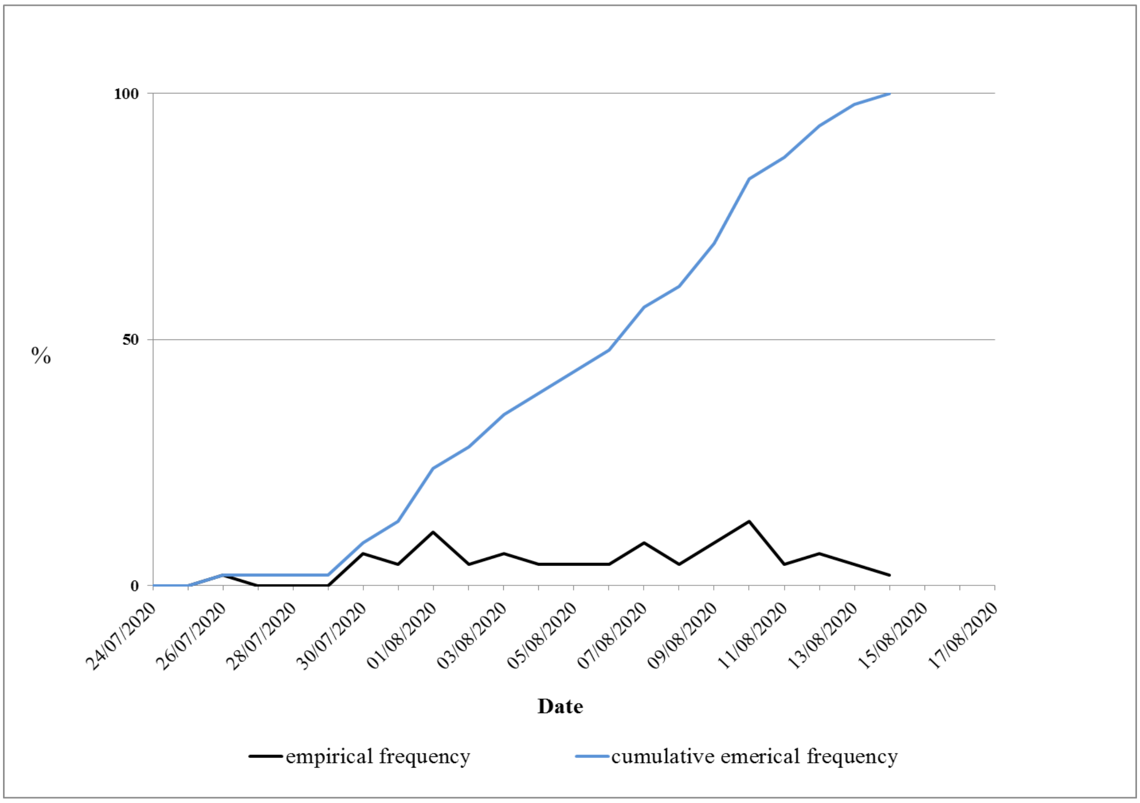

Fig. 1. Dynamics of the hatching of egg batches from Dobrostan (summer form of PPM).

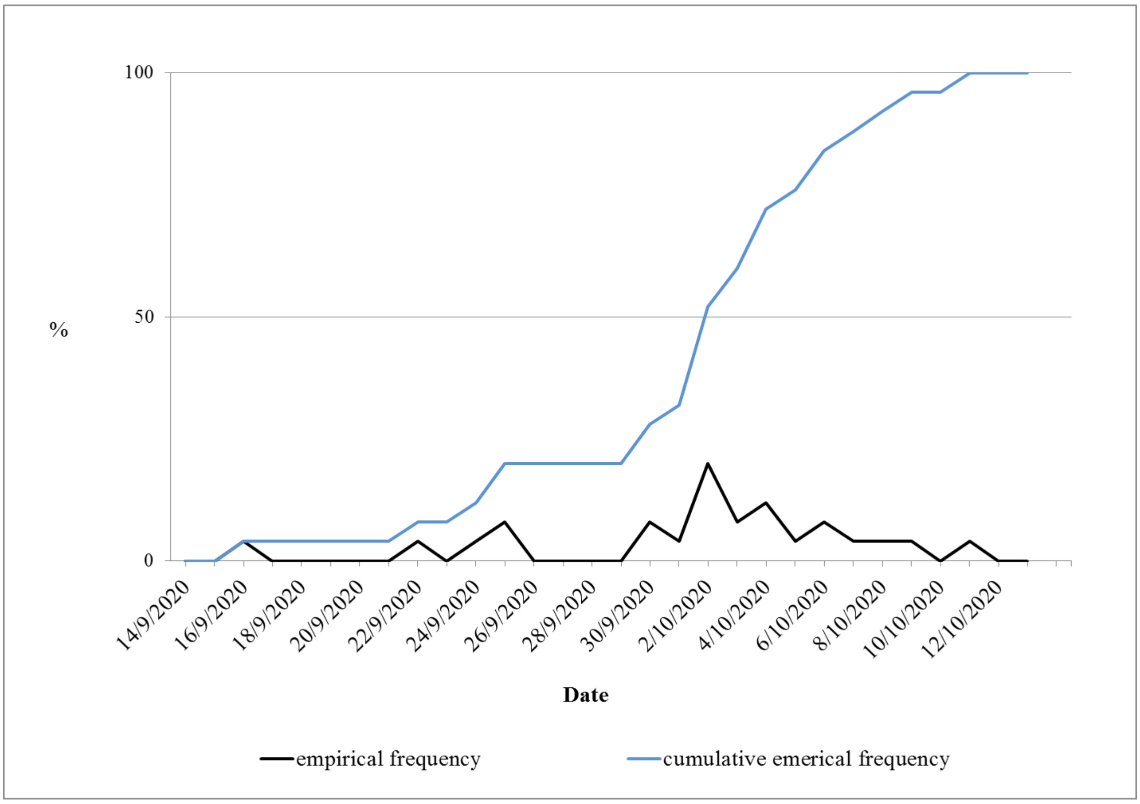

Fig. 2. Dynamics of the hatching of egg batches from Sandanski (winter form of PPM).

In Figures 3 and 4 the hatching period of egg batches from Dobrostan and Sandanski has been plotted together with the average monthly maximum air temperatures of the region of origin. The hatching period is given by the hatching of the first and the last egg-batches for the season (absolute hatching period), as well as by its peak determined the 50-th percentile. In the hottest region of the country – Sandanski, the PPM hatching took place well after the hottest time of the year and its peak came in a moment, when the temperatures did not exceed 28°C (Fig. 3), which is well below the threshold of 32°C known as lethal (Battisti et al, 2015). On the opposite, the hatching of larvae of the summer population in Dobrostan occurred during the hottest period (July and August), but in this region the average monthly maximum air temperatures did not exceed 28°C nevertheless (Fig. 4).

Discussion

Thaumetopoea pityocampa is one of the most common defoliator insects found in pine plantations in Bulgaria. Its heavily attacks for the period 2003-2018 reach 2700 ha per year (Zaemdzhikova et al., 2019). Significant money is spent each year to combat its attacks, thus preventing losses of growing stock and increment. In Bulgaria, so far no monetary valuation of the damage from PPM has been made, but other countries' studies show serious monetary value of annual increment and growing stock losses caused by strong attacks of pine processionary moths (CABI, 2020). Regardless of their relatie merits, both criteria – the price of control measures and the amount of losses, calculate significant moneys. In this regard, it is important to take into account that the phenological calendar of the pest is of great importance for the effectiveness of control measures: In the case of PPM, in order to maximize the effect of control measures, they should be applied against the caterpillars of the first and second larval stage, when the young larvae have not yet created dense nests.

The phenological cycle of *T. pityocampa* is strongly influenced by the temperature regime of the habitats. Temperature thresholds are crucial for the emergence of adults and larvae, and are the primary regulator of the duration of the pupal diapause (Robinet et al., 2013; Salman et al., 2016). It is known that in the south and at low altitudes, the adults of the PPM fly in autumn avoiding high summer temperatures, while in the north and at high altitudes they fly in summer (Robinet et al., 2013). French publications usually refer to the winter form of PPM, while in Bulgaria things are complicated by the very widespread distribution of summer form. In Bulgaria, however, regardless of the phenological forms, the flight of moths is during the hottest months of the year, culminating before the peak of the heat waves. In the most observed cases, the flight of PPM is quite long – two or three months and in some places even five or six months. There is a great local variety of the flight period, but in the places inhabited by the summer form, the flight begins and ends a month and a half earlier (Zaemdzhikova, 2020; Zaemdzhikova et al., 2020b). Given the mountainous relief of the habitats, characterized by strong variations in altitudes, as well as the flying abilities of males up to 50 km (Mirchev et al., 2013), it is quite possible that individuals from distant populations are present in the catch of one pheromone trap. Distant populations higher of lower in the mountain may have quite different calendars than the local population and may even belong to a different phonological form. The participation of distant populations may explain the extended period of flight in most Bularian sites regardless of the established local phonological form usually established y using pheromone traps. For this reason, for a country like Bulgaria, the use of pheromone traps as a means of monitoring the flight is quite uncertain and can not be used to identify the phonological form. This calls into question and its suitability, as a monitoring tool, in predicting the emergence of young caterpillars.

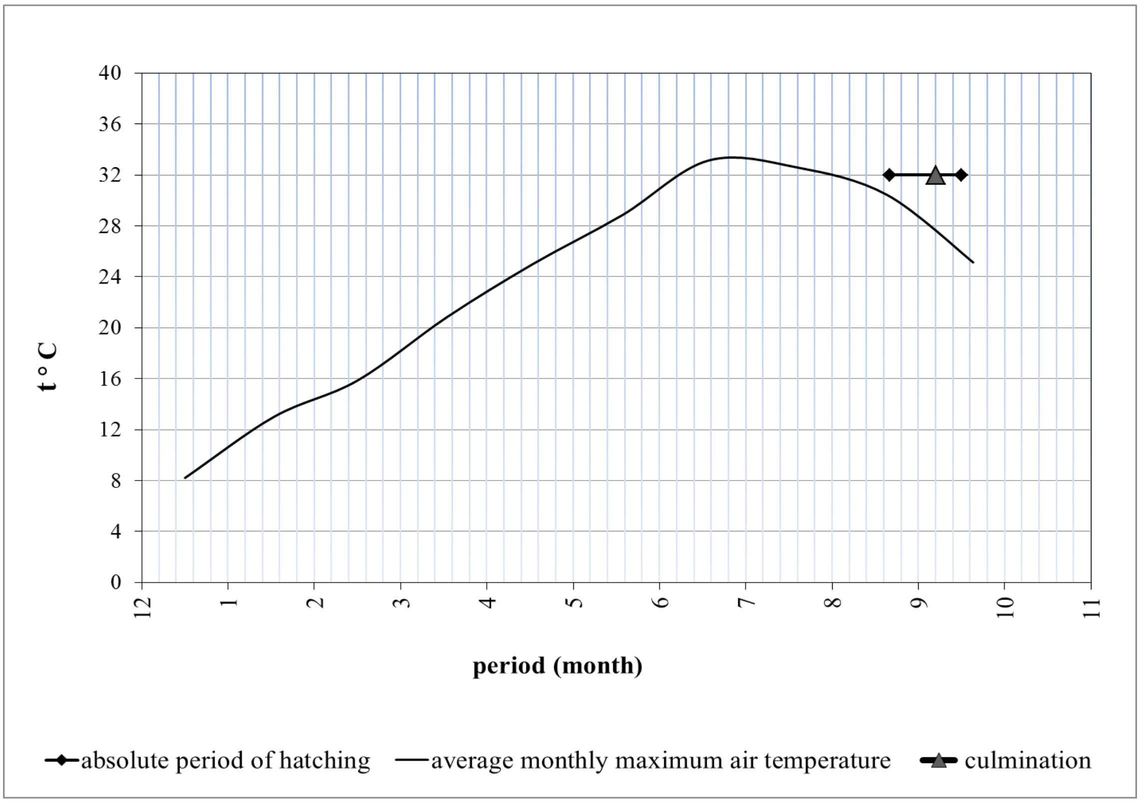

Fig. 3. Comparison of the hatching period and its peak with the temperature profile in Sandanski.

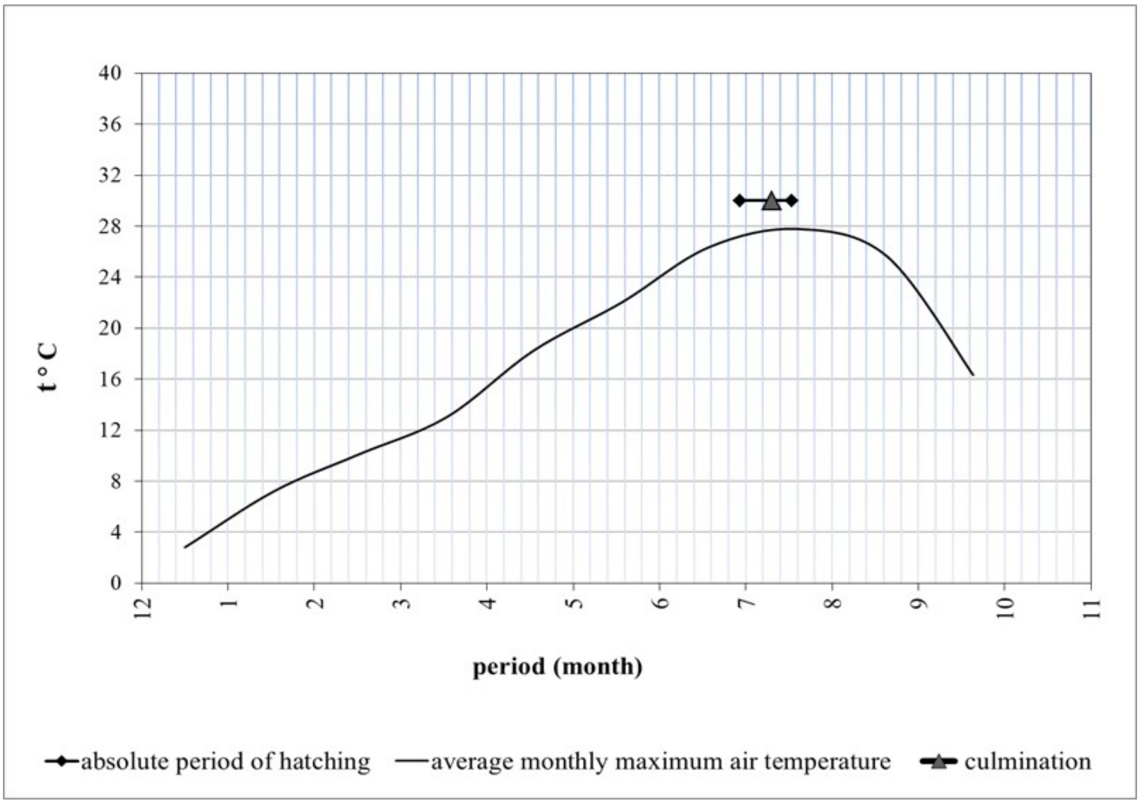

Fig. 4. Comparison of the hatching period and its peak with the temperature profile in Dobrostan.

The result of the present study shows, that the established hatching dates of the caterpillars correspond to the phenological calendar of the two populations (winter type or summer type calendar), as well as to the temperature thresholds limiting their survival. In Sandanski, the development of the larvae is within the known temperature optimum, with the culmination of hatching after the summer extremes. Of course, delaing the hatching after the hot waves there in July and August does not save the WF from all effects of heat since hot wawes have a negative effect on the embryonic stage of PPM. In the literature, the temperature maxima above 42°C which are reported periodically in Sandanski (Zaemdzhikova et al., 2020b) are considered lethal for development of the embryos of PPM (Robinet et al., 2013; Rocha et al., 2017). A higher percentage of unhatched dead caterpillars in the winter form – 7.3% against 3.2% in the summer form was proved for the Bularia in a study by Mirchev et al. (2020). In the region of Dobrostan, due to the mountainous terrain and the high altitude (over 1000 m a.s.l.) no heat waves are registered. In our records, the hatching of caterpillars from Dobrostan egg batches began in late July, culminating in early August, similar to the already known hatching period in the region of Kirkovo (Georgieva et al., 2019).

Considering the potential effects of the future climate warming scenarios, scientific considerations suggest that the southern regions will become less favorable for winter populations of *T. pityocampa*, as a result of the expected increase in summer extremes, which will increase the risk of the embryo survival. This may be the reason for the reduction of PPM attacks in Southern Bulgaria, found by (Zaemdzhikova et al., 2020a). On the other hand, global warming may be causing a shift in the phenological calendar of the winter form to the winter months, which is observed compared to the data from the 1950s. In more recent times For the region of Sandanski, Tsankov & Mirchev (2000) reported the beginning of the larvae hatching of winter populations in 2000 on 8th September with a culmination on 15th September, which is two weeks earlier than the established deadlines set by us in 2020. A quite probable explanation of this difference is that during a period of twenty years a shift in the phenology calendar of winter populations in their southern border has occurred, which is an expected result of global warming. The survival of the larvae of the WF seems to require a shift of the flight period towards the winter months.

The influence of temperature thresholds in Bulgaria is complicated by the orography and the presence of phenologically different populations, In addition to the climatic region and altitude, temperature extremes are strongly influenced by other environmental factors that enhance or mitigate the local impact of the temperatures. Such are: the relief forms, the exposure, the proximity of water area, etc. For instance, the towns Kirkovo and Sandanski are located at equal altitudes (average 300 m a.s.l.). Nevertheless in Kirkovo the SF is present, while in Sandanski – it is the WF (Mirchev et al., 2019). This difference is obviously due to the terrain forms that model the microclimate in these habitats. Kirkovo is located in a hollow field that keeps the cold air masses. For this reason in Kirkovo winters and summers are cooler. The minimum temperatures in Kirkovo are often from -10 to -12°C, and in some cases -24 ÷ -26°C while in the Petrich-Sandanski climatic region the minimum temperatures fall to -8 ÷ -9°C and in extremely cold weather can drop to –18÷ -19°C (Georgieva et al., 2018). The cold winters in Kirkovo are probably an explanation for the presence of the summer populations there.

Another example deliver the villages Dobrostan and Muldava also inhabited by phenologically different populations. The distance between them is 10-12 km as the crow flies. Both villages are assigned to the continental-Mediterranean climate area (Sabev & Stanev, 1959). In the land of Muldava the winter form is found, and in Dobrostan – the summer form. Due to the serious difference in altitude of about 800 m a.s.l. (Mirchev et al., 2019), the temperature thresholds in them should be quite different. In the higher site Dobrostan (1110 m a.s.l.) it is assumed that the winters will be colder than in Muldava (300 m a.s.l.) and conversely the summers in Muldava will be hotter than Dobrostan. In the first case (Sandanski – Kirkovo) the determining factor for the phenological form is the oreography, and in the second (Dobrostan – Muldava) the determining factor is the altitude.

Conclusions

While the flight of PPM in Bulgaria lasts several months, usually two or three, sometimes even five or six, the hatching of the larvae takes a much shorter period – in our case it took about 3 weeks. This supports the assumption that pheromone traps catch both specimens from the local population and from remote ones, which makes it difficult to establish the phenological calendar of the local population.

Our case study in Sandanski and Dobrostan confirms with new data the observation that in Bulgaria the development of the winter form follows with a delay of a month and a half the development of the summer form.

The larvae hatching of the winter forms of PPM culminates in the beginning of October and occurs after the summer heats. On the other hand, the culmination of the summer form’s hatching ocurs in early August and coincides with the summer heats. In both cases, however, the culmination of hatching occurs at average monthly maximum air temperatures of about 28°C.

In the phenological calendar of the winter form there is a change in the hatching period as compared to 2000, which is a supposed effect of global warming.

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