

## Structure and Parasitism of Egg-Batches of the Pine Processionary Moth, *Thaumetopoea Pityocampa* in the Algerian Cedar Forests.

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### Cover Page Footnote

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## STRUCTURE AND PARASITISM OF EGG-BATCHES OF THE PINE PROCESSIONARY MOTH, THAUMETOPOEA PITYOCAMPA IN THE ALGERIAN CEDAR FORESTS.

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### ABSTRACT

Today, climate change is one of the greatest threats facing forest ecosystems. It directly influences the geographical distribution of insects and increases epidemics of harmful insects. The present work took place in this context. It aimed to contribute to the knowledge of egg parasitoids that could affect the evolution of the pine processionary caterpillar *Thaumetopoea pityocampa*, a major forest pest in Algeria and Mediterranean countries. The study was carried out on 120 batches of eggs taken from the Atlas cedar at two sites in the Chelia cedar zone (case of eastern Algeria) in 2017. It allowed the analysis of a total of 37,943 eggs. At the first site, the hatch rate was 66%; the egg mortality rate increased at a rate of 15 %, and the parasitism rate was rather high at 18%. The second site was characterized by the parasitism rate lower by about 11%, while the egg mortality rate was relatively comparable in the order of 14% with a hatching rate of 75%. The eggs are more parasitized by *Baryscapus servadeii* (79%) than by *Trichogramma embryophagum* (17%), while *Ooencyrtus pityocampae* remains negligible (4%). Egg parasitoids are an important killer of *Thaumetopoea pityocampa* eggs.

**Key words:** *Thaumetopoea pityocampa*, egg batches, Atlas cedar, egg parasitoids, Algeria.

### INTRODUCTION

The Atlas cedar (*Cedrus atlantica* Manetti) is a species native to Northern Africa. It grows in the high mountain peaks of Morocco, at an altitude of over 2,000 m over an area of 130,000 ha. In Algeria it spans an area of 27,000 ha at altitudes between 1,000 and 2,700 m (Benabid, 1994; Terrab et al., 2008). This forest species is one of the most economically and ecologically important in the Mediterranean mountains (Terrab et al., 2006).

In Algeria, cedar stands are formed in the Chelia massifs and spread over extensive areas. These forest formations are now threatened by man-made degradation and the impact of climate change, combined with other hazards such as attacks from

different pests like defoliating insects, especially processionary moth (*Thaumetopoeidae*) (Bentouati and Bariteau, 2006).

Two species of processionary attack the Atlas cedar of Algeria. The winter processionary moth *Thaumetopoea pityocampa* affects cedar and all pine species. The summer processionary moths *Thaumetopoea bonjeani* are specific to the Atlas cedar and differ from *T. pityocampa* by their larval development in the spring and early summer and the absence of winter nests (Tsankov et al., 1995; Rahim et al., 2016).

The pine processionary moth (*T. pityocampa* Schiff) is the main defoliator of resinous forests throughout the Mediterranean Basin (Robinet et al., 2011). This defoliator eliminates photosynthetic organs, affecting many

vital functions and in turn, disrupts the growth and can even lead to the death of the tree (Kanat et al., 2005; Allen et al., 2010). Trees weakened by severe and repeated defoliation are more susceptible to attacks by secondary pests and pathogens (Hedar et al., 2003; Carus, 2004; Battisti et al., 2005; Robinet et al., 2007; Jacquet et al., 2012). They therefore cause considerable forest damage of not only an economic but also ecological nature. Additionally, this defoliator has microscopic urticating hairs that are very allergenic and which can potentially cause violent health reactions in both humans and domestic animals (Maier et al., 2003; Battisti et al., 2011).

The ecological and health consequences of the presence of the pine processionary moth justify the establishment of various means of control against this pest, particularly chemical and microbiological. The latter—based on *Bacillus thuringiensis*—is currently the most used against this forest pest (Chenchouni et al., 2010). Despite their effectiveness, these control methods disrupt the environmental ecology of the forest. The pine processionary moth is under pressure from many natural enemies in all ecophases of their development cycle, namely predators, pathogens, and global parasitoids (Masutti and Battisti, 1990; Battisti et al., 2000). The egg parasitoids of this defoliator are more frequently used to effectively control this pest as part of environmentally friendly biological control (Zovi et al., 2006; Jactel et al., 2015).

Several research projects focusing on several factors, namely, biology, ecology, and damage, have been carried out in Algeria. However, research on the parasitic complex of the defoliator *T. pityocampa* in Algeria has been limited up to this point. This work aimed to study some egg batch parameters of the pine processionary moth (*T. pityocampae* Schiff) on the Atlas cedar at the Chelia

#### ***Methodology of the Study***

mounts in the Aures forest -eastern Algeria and to identify the egg parasitoids of this pest.

## **MATERIALS AND METHODS**

### ***Study Area***

The massif of Chelia is in the southeastern part of the city of Batna, in the easterly direction of Aures, and spreads over approximately 8,000 hectares (Bentouati, 2008). The massif is primarily afforested with cedar and is characterized by rugged terrain with several mountains of altitudes ranging from 1,600 – 2,328 m.

Soils found in the Chelia mountains contain sediments derived from ferruginous sandstones mixed with those of limestone and dolomite, both of which date from the Lower Cretaceous period (Bentouati, 2008). The forests of Chelia are characterized by a Mediterranean climate alternating, between two contrasting seasons: cold and humid, and hot and dry. The annual median rainfall in Chelia is approximately 480 mm. In this context, Chelia mounts is highly vulnerable to current climate change, the analysis of climate reveals an increase in temperature, a decrease in rainfall and more frequent droughts.

The dieback of cedar has particularly affected old trees in the forests due to poor water supply (Bentouati and Bariteau, 2006). Today, even younger cedar trees are getting affected similarly.

Sample sites and localities are chosen for their altitude and relatively homogeneous populations of the Atlas Cedar. The characteristics of the prospected stations are recorded in Table 1

**Table 1: Characteristics of the study sites.**

<b>Sites</b>	<b>Altitude (m)</b>	<b>Exposure</b>
<b>01</b>	1930	East
<b>02</b>	1830	West

After *T. pityocampa* caterpillars emerged, 120 egg batches were gathered at an accessible height in September and October 2017 at both sites, which are at a relatively high altitude. Every egg batch was collected from a different tree the plant guests of the pine processionary moth are the Atlas cedar trees in order to avoid differences between populations that occur owing to the host plant (Arnaldo and Torres 2006).

The length of the egg batches was measured with an electronic caliper. The biological material was separately stored in glass tubes (10 x 1 cm), sealed with cotton on both sides to maintain ventilation and laboratory conditions at a room temperature of 26°C (Schmidt et al., 1999). Additionally, almost daily observations were performed and the emergence of parasitoids was identified and counted (Schmidt et al., 1997).

After the emergence of parasitoids, the scales covering the egg batches were removed to count the different categories of eggs in every egg batch using a binocular loupe; the count is based on the diameter of exit holes.

The meconium of *Ooenchrtus pityocampae* is a yellow flat disc, while the meconium of *Baryscapus servadeii* resembles a single ball stuck to an eggshell. *Trichogramma embryophagum* recognized by a small round hole formed in the eggshell (Schmidt and Douma-Petridou 1989).

We established five categories of eggs:

- 1 – Hatched eggs.
- 2 – Unhatched eggs.
- 3 – Empty eggs.
- 4 – Parasitized eggs.
- 5 –predated eggs.

We were allowed to gain data on the total number of eggs of the pine processionary moth, the hatching of caterpillars, the natural mortality of caterpillars at the embryonic stadium, and

the total parasitism of oophagous parasitoids.

### **Statistical Analysis**

The statistical analyses were accomplished with SPSS 25. A descriptive analysis was used to calculate averages, standard deviations, and the coefficient of variation to determine a significant difference between the mean values. Simple linear regression tested the relation between the number of eggs and the size of the egg batches. The test also performed a comparison of the averages of the measurements of the length of egg batches and the enumerations of eggs between both stations. A one-way ANOVA analysis of a comparison of averages was performed to test the difference between the parasitoids of the pine processionary moth in both stations.

## **RESULTS**

### **Analysis of Biological Material**

The 120 egg batches taken from the Atlas cedar tree and in sites 1 and 2 allowed the analysis of 37,943 of eggs total, with an average of 325 and 308 eggs per batch from Sites 1 and 2, respectively. The first site suggests a greater fecundity of the females of *T. pityocampa* exists in comparison to the second site.

Additionally, the eggs gathered from Site 2 have a significantly smaller diameter and length than those taken from site 1.

Figures 1 and 2 represent a comparison of the averages of length, diameter, and the number of eggs in the egg batches at both sites.

Box plots show the five-number summary of a set of data, including the minimum score, first (lower) quartile, median, third (upper) quartile, and maximum score. It can tell you about outliers and their values.

The results show a highly significant difference between the lengths of egg

batches from each site ( $p = 0.008$ ) Figure 1. As well as a highly significant difference for the diameter of egg batches ( $p = 0.000$ ) figure 2. On the contrary, the comparison of the number of eggs from the sites does not show a significant

difference ( $p = 0.259$ ). In the Chelia series, the dynamic potential of the laying females of *Thaumetopoea pityocampa* is higher figure 3.

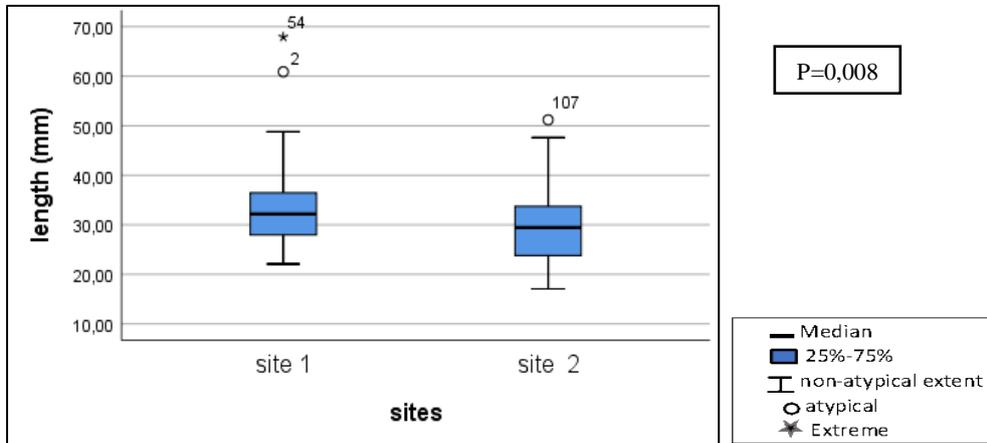


Figure 1: Comparison of the average lengths of the egg batches from both sites.

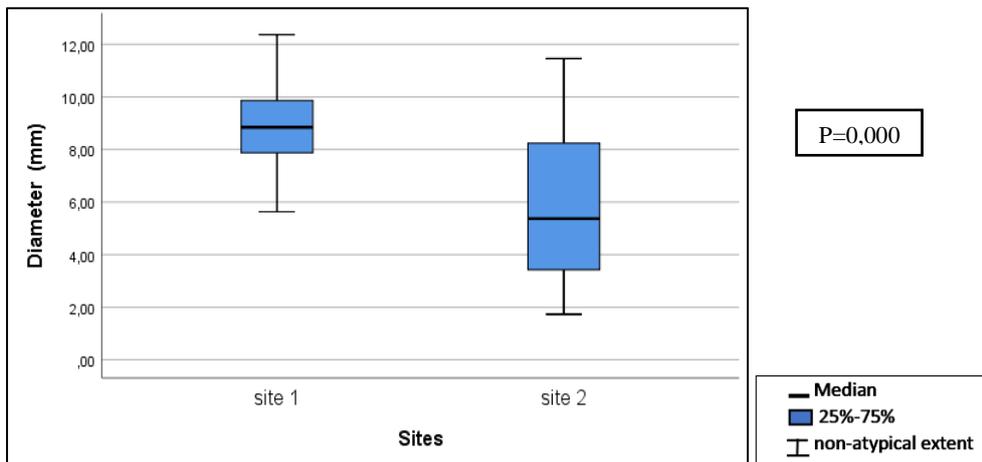


Figure 2: Comparison of the average diameters of the egg batches from both sites.

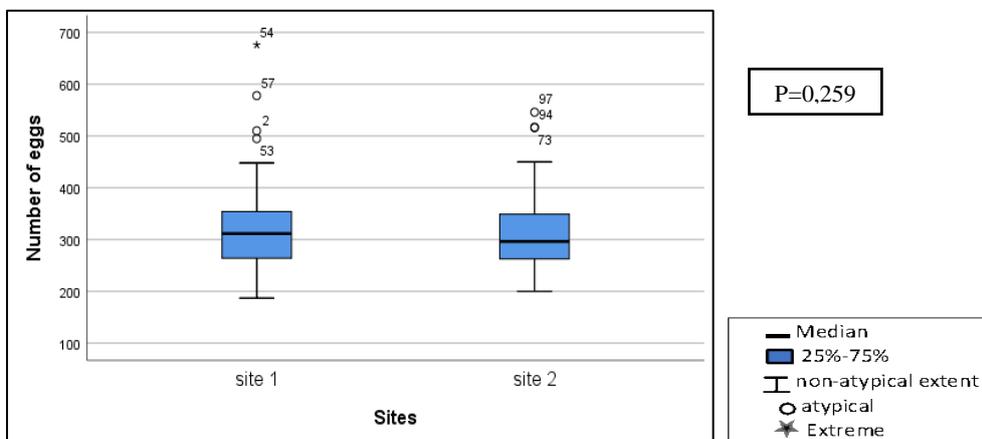
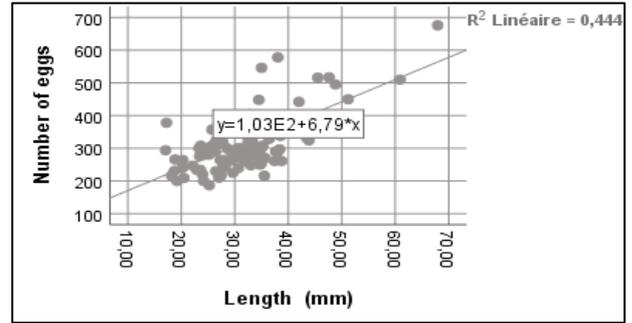


Figure 3: Comparison of the average number of eggs from both sites.

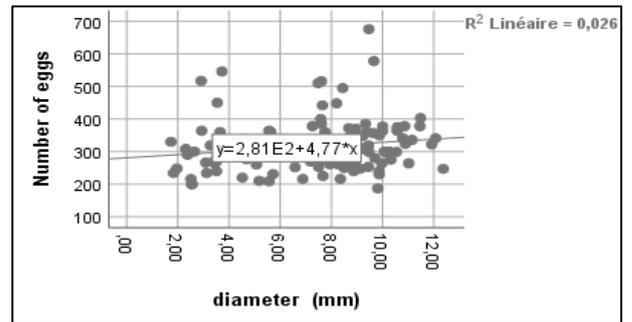
**Relationship between Biometric of Egg Batches and the Number of Eggs**

The relationship between the length and diameter measurements and the number of eggs is recorded in Figs 4 and 5. Linear regression analysis reveals a relation between the number of eggs and the length of egg batches with a high significant probability ( $p = 0.000$ ) and a coefficient of determination corresponding to  $R^2 = 0.44$  figure 4. Linear regression analysis shows a relation between the diameter of egg batches and the number of eggs with a high significant probability ( $p = 0.000$ ).

The results indicated a relationship between the lengths and diameters of the egg batches and the number of eggs with a high significant probability ( $p = 0.000$ ) between these parameters.



**Figure 4: Relationship between the number of eggs and the length of the egg batches.**



**Figure 5: Relationship between the number of eggs and the diameter of the egg batches.**

**Table 2: Descriptive analysis of the categories of eggs.**

Site	Hatched eggs			Unhatched eggs			Parasitized eggs		
	Total Number	Mean (±SD)	Extreme Values	Total Number	Mean (±SD)	Extreme Values	Total Number	Mean (±SD)	Extreme Values
Site 1	12,931	215.52 ± 79.05	31-432	2,982	49.70 ± 70.43	9-561	3,460	57.67 ± 31.49	4-137
Site 2	13,900	231.67 ± 84.80	11-465	2,649	44.15 ± 33.64	6-185	2,053	34.22 ± 27.24	1-123

**Analysis of the Categories of Eggs**

Table 2 shows the data on the enumeration of the categories of eggs. The average number of eggs hatched varies from 216 on average at site 1 to 232 at site 2, and the number of eggs hatched at site 2 is more consequential than those at site 1. The number of unhatched eggs varies from 50 on average at site 1 to 44 at site 2. We note that site 2 is characterized by the number of unhatched eggs smaller than at the first site. Finally, the parasitized number of

eggs varies on average from 58 to 34 at sites 1 and 2, respectively. As a result, the egg batches parasitized at site 1 are more important than those parasitized at site 2.

A student's t-test was also performed to compare the hatched eggs for the independently acquired samples (Table 3).

**Table 3: Tests T of Student for hatched eggs unhatched and parasitized by site.**

Test of the	independent samples					
	Test t for equality of averages					
	t	Sig. (tailed)	Mean difference	Std. Error Difference	95% Confidence Interval of the Difference	
					Lower	Upper
<b>Number of hatched eggs</b>	-1.079	.283	-16.150	14.967	-45.788	13.488
<b>Number of unhatched eggs.</b>	.551	.583	5.550	10.077	-14.405	25.505
<b>Total number of parasitoids</b>	4.363	.000	23.450	5.375	12.804	34.096

**Table 4: Egg parasitoids of the pine processionary moth.**

Parasites	Site 1 (60)			Site 2 (60)		
	Number of parasitized egg batches	Number of parasitized eggs	Percentage %	Number of parasitized egg batches	Number of parasitized eggs	Percentage %
<i>Baryscapus servadeii</i>	52	1,298	6.67	31	531	2.87
<i>Trichogramma embryophagum</i>	25	144	0.74	32	252	1.36
<i>Ooencyrtus pityocampae</i>	12	20	0.10	22	71	0.38

Results show that there is not a statistically significant difference ( $p = 0.283$ ) between the averages of eggs hatched at both sites at a level of confidence of 95%. It is important to note that the total number of eggs is higher at the level of site 1, corresponding to colonies of the pine processionary more densely populated than in site 2. We have not found a significant difference ( $P = 0.583$ ) between the averages of eggs unhatched at both sites to a level of confidence of 95%. Since the value of probability is  $p = 0.000$ , which is less than 0.05, there is a very highly significant difference between the averages of eggs

parasitized between sites to a 95% confidence level.

#### **Embryonic Parasitoids**

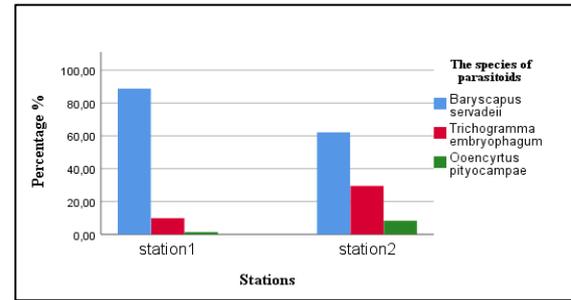
Three species of parasitoids belonging to Chalcidoidea (Hymenoptera) parasitizing eggs of the pine processionary moth in the Chelia mount *B. servadeii*, *O. pityocampae*, and *T. embryophagum* (Table 4). The specialist *B. servadeii* is the majority species and is found throughout the study area (Figure 6).

At site 1, *B. servadeii* represents 1,298 parasitized eggs belonging to 52 egg

batches, or a rate of 6.67 % against 31 egg batches was noted, or 531 eggs parasitized at a rate of 2.87 % per egg at site 2 (Table 4).

*T. embryophagum* demonstrates a rate of parasitism that varies on average from 0.74 % at site 1 to 1.36 % at site 2. The latter site shows greater rates of infection of these parasitoids (Table 4). Finally, *O. pityocampae* is in weak abundance in a proportion that remains very restricted. This species has a negligible impact on the processionary of the pine in the Chelia massifs (Figure 6). As the probability value is equal in  $p = 0.000$  for the F test, which is less than 0.05, a very highly significant difference between the averages of different species

of parasitoids of a level of the site to the other one Table 5.



**Figure 6: Rate of parasitism of the egg parasitoids of *T. pityocampa* identified on the Atlas cedar in Chelia.**

To draw more information on parasitoids, an ANOVA was carried out (Table 5).

**Table 5: ANOVA for egg parasitoids by site.**

		Sum Squares	of df	Mean Square	F	Sig.
<i>Baryscapus servadeii</i>	Between Groups	101034.411	1	101034.411	100.517	.000
	Within Groups	1836403.325	1827	1005.147		
	Total	1937437.735	1828			
<i>Trichogramma embryophagum</i>	Between Groups	7642.601	1	7642.601	197.312	.000
	Within Groups	70766.139	1827	38.34		
	Total	78408.740	1828			
<i>Ooencyrtus pityocampae</i>	Between Groups	344.222	1	344.222	169.048	.000
	Within Groups	3720.210	1827	2.036		
	Total	4064.432	1828			

## DISCUSSIONS

Among possible disturbances to forest ecosystems, the damage caused by *T. Pityocampa* is one of the most significant causes of the decrease in forest productivity. Increased outbreaks of these insects have seriously affected forest health. The analysis of the pine processionary moth egg batches from two sites revealed the impact of egg parasitoids on the processionary moth populations.

The descriptive analysis of the measurements of the egg batches gathered in the forests of Chelia showed a consequential significance for the lengths and diameters of the egg batches; those collected from site 1 were larger than those from site 2. Arnaldo and Torres (2006) revealed a difference in the morphology of the egg batches of the pine processionary that may be affected by the host. The host plant plays a decisive role in the egg

batches' size and structure and determines how and where the eggs mass.

The egg batches in both locations contained a very large number of eggs. These results show the year (2017) is marked by a very high processionary population. In the sites in question, the females' fertility rate is substantial. According to Myers (2000), fluctuations in Lepidoptera fertility are associated with fluctuating population density, the high reproductive potential of the pine processionary moth can have significant consequences on the increase in the density of the pine processionary moth and can cause tree defoliation.

Cross-analysis reports show a relationship between egg mass, length and diameter, and egg counts with a highly significant probability at both prospected stations.

Zamoum et al., (2017) showed that the morphology of the host plant has a major influence on the fertility of females in their natural habitats. All the environmental conditions of the host plant will profoundly affect fertility; the latter could also be more sensitive to climatic conditions, which would reduce their populations in colder regions.

The average number of eggs per egg batch represents the overall reproductive efforts of the female (Battisti et al., 2015). The rate of hatching of the caterpillars is lower in station 1 (66 %) than in station 2 (75 %). The rate of hatching is mainly affected by two factors: natural mortality in the embryonic stages and the impact of natural enemies.

The natural embryo mortality increased at a rate of 15 % in station 1 and 14 % in station 2. Imbert (2012) notes that the hatching rate varies on average from 65.26 % to 96.73 % and the mortality rate varies on average from 2.82 % to 12.24 % on samples of egg batches of the pine processionary in France.

Comparatively, the parasitism rate was quite high, with a rate of 18 % in site 1 and 11 % in site2. Thus, the egg batches

from area 1 populations had a significantly higher rate of parasitism than in zone 2.

Embryonic parasitoids are among the important natural regulators for the populations of the processionary moth *T. pityocampa*, in particular, can have a significant effect on the density of host species (Tiberi, 1990; Mirchev et al., 1998).

We found three species of parasitoids parasitizing eggs of pine processionary moth on this massif: *B. servadeii*, *O. pityocampae*, and *T. embryophagum*.

The specialist species *B. servadeii* is the majority species and is found in the whole of the Chelia series with a rate of 79 %. *Thaumetopoea* is mainly parasitized by the parasitoid monophagous *B. servadeii* throughout the Mediterranean basin (Auger-Rozenberg et al., 2015). The species *B. servadeii* is considered the specialist species and *O. pityocampae* as the generalist in both sites prospected.

The polyphagous species *O. pityocampa* is noted as having a low abundance of 4%. This species, although it is a hyperparasitoid, is characterized by a low parasitic incidence, which is believed to be due to barriers from the protective scales of the egg batch of *T. pityocampa* (Palmeri and Pulvirenti, 2004).

In Italy and Greece, it is *B. servadeii* that is more abundant than *O. pityocampae* (Tsankov et al., 1999; Zovi et al., 2006), unlike in Turkey, where it is *O. pityocampae* that is more abundant than *B. servadeii* (Mirchev et al., 2007).

Existing studies of pine processionary parasitoids have always highlighted the predominance of two species of parasitoids, the specialist *B. servadeii* and the generalist *O. pityocampae*, with varying proportions depending on the region. However, during our study, the combinations of the presence of three parasitoids are noted on the biological material examined, with a dominance of two species: *B. servadeii*, and *T. embryophagum*.

A parasitic efficacy in the polyphagous *T. embryophagum* was noted in the Atlas cedar forest of mount Chelia, representing a rate of 17 % of the total parasitoids. This species is a polyphagous endoparasitoid, and characterized by a polyembryony. Over 10 individuals can develop in one egg (Zamoum et al., 2017), which helps to destroy a large part of the host's eggs. Mirchev et al., (2007) note that in Turkey, *T. embryophagum* has been observed with a parasitism rate that varies on average from 0.3 % to 21.8 % depending on the region where the egg batches were collected. Furthermore, in Bulgaria, studies show a higher percentage of *T. embryophagum* (Mirchev et al., 2017).

Other species have also been reported, but their impact is often considered negligible.

The parasitism of the pine processionary moth eggs are a complex process, depending on many factors (Mirchev et al., 2021).

Scales are regularly placed on the surface of egg, to play a protective role for the eggs against the actions of various natural enemies, including parasitoids as well as others. This can reduce both the influence of parasitoids and their distribution on the surface of the eggs of the processionary moth populations.

Climate parameters are among the ecological factors that influence insect populations in their biotope. The composition of parasitoid species and their number are determined, to a large extent, by ecological conditions in various biotopes.

## CONCLUSION

The Atlas cedar *Cedrus atlantica* is one of the most important resinous trees in northern Africa; economically, aesthetically, and culturally. This tree has been in decline over the past two decades due to the phenomenon of worrying dieback that has settled because of

interaction with several destructive factors. Among others, the winter pine processionary moth *T. pityocampa* (Lep., Thaumetopoeidae) is a redoubtable defoliator of the Atlas cedar. Climate plays a key and critical role in geographic distribution, development, and the abundance of this species and their parasitoids.

In our study, the gathered data showed that the effect of altitude and exposure on the spatial distribution of the pine processionary moth is clearly significant in Chelia. The selection of host trees by the winter pine processionary moths involves a large number of chemical and physical cues that act on different spatial and temporal scales (Jactel et al., 2015).

Egg parasitoids of processionary moths are essentially Hymenoptera of the chalcid family used for regulating *Thaumetopoea* populations. These parasitoids are a biologically controlled approach that can be very effective, following the ecological rules of stability and balance.

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