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PUPA MORTALITY OF *ORGYIA TRIGOTEPHRAS* BOISDUVAL, 1829 (EREBIDAE LYMANTRIINAE) IN TUNISIA

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Hammami S., Ezzine O., Dhahri S., Villemant C., Schmidt S., Ben Jamâa M.L. – Pupa mortality of *Orgyia trigotephras* Boisduval, 1829 (Erebidae Lymantriinae) in Tunisia.

Orgyia trigotephras (Boisduval) is a polyphagous moth which is bivoltine in Tunisia, with two generations (spring and autumnal). This work, carried out in northeastern and northwestern Tunisia, aimed at studying pupal mortality of this pest on two host species, *Quercus coccifera* L. and *Pistacia lentiscus* L. Two types of antagonist factors were assessed: natural mortality (dried pupae) and mortality caused by parasitoids. Three pupal parasitoids species belonging to Hymenoptera order were identified: *Brachymeria tibialis* (Walker), *Pimpla rufipes* (Miller) and *Monodontomerus minor* (Ratzeburg).

KEY WORDS: *Orgyia trigotephras*, *Quercus coccifera*, *Pistacia lentiscus*, Parasitoids, Tunisia.

INTRODUCTION

Populations of forest pests often fluctuate, showing outbreaks and extended periods of low and endemic densities (BERRYMAN *et al.*, 1987). Phytophagous insects may select plants or plant parts not only based upon nutritional content, but also on the intensity of predation and parasitism they may undergo (BERNAYS *et al.*, 1988). During outbreaks, Lepidoptera defoliators of *Quercus* trees cause considerable damage, often leading to complete defoliation of trees. This defoliation could cause negative effects on their production and regeneration. Dynamic of lepidopteran insects is limited by the effect of the quality and the quantity of foliage of the host plant (SCHULTZ & BALDWIN, 1982; FRAVAL, 1984; MATTSON & HAACK, 1987), favorable climate (MARTINAT, 1987) and parasitoids (CANDAU, 2008) which are generally considered as key natural enemies (KIDD & JERVIS, 1997). These parasitoids have an important place in the ecosystems notably as natural control agents of herbivorous insects (GODFRAY, 1994).

Orgyia trigotephras is a polyphagous moth widely distributed across the Mediterranean Basin (BASRI, 1994). It feeds on plants of the genus *Quercus*: *Q. suber* L., *Q. ilex* L. (VILLEMANT & FRAVAL, 1993; CHAKALI *et al.*, 2002) and *Q. coccifera* L. (EZZINE *et al.*, 2010), but also on shrub species, mainly: *Pistacia lentiscus* L. (EZZINE *et al.*, 2010; BELLA *et al.*, 2011) and *Retama monosperma* L. (DIONISIO, 2002).

Populations of oak lepidopteran pests are regulated by a variety of parasitoids that attacks every of their preimaginal instars (VILLEMANT & FRAVAL, 1991). In Morocco, in the Mamora cork oak forest, VILLEMANT (1989) reported the presence of five species of Hymenoptera parasitizing

Lymantria dispar pupae: The Ichneumonidae *Pimpla rufipes* (F.), *P. turionellae moraguesi* (Schmiedeknecht) and *Vulgichneumon* sp., as well as two species of Chalcidoidea: the Chalcididae *Brachymeria tibialis* (Walker) and the Torymidae *Monodontomerus aereus* (Walker). Among them *P. turionellae moraguesi* and *M. aereus* were also reported as parasitoids of *O. trigotephras* in the same forest (VILLEMANT & FRAVAL, 1991). BASRI (1994) also reported that *P. turionellae moraguesi* and *P. rufipes* attack both moth species in Morocco.

In a previous work (HAMMAMI *et al.*, 2017) we showed that larval mortality of *O. trigotephras* in Tunisian oak forests has a significant impact on its population dynamics. In the present work, we combined field data and laboratory experiments, to evaluate the factors inducing pupal mortality of this pest: (i) dried pupae and (ii) mortality due to pupal parasitoids.

MATERIALS AND METHODS

STUDY AREA

The study was carried out in the northeastern (Jebel Abderrahmane, Cap Bon) in (Ftahiz; alt. 121m, 36°52'N, 10°45'E), (Delhiza; alt. 401 m, 36°51'N, 10°47'E) and (Guitoun; alt. 136m, 36°83'N, 10°82'E) and the northwestern (Sejnane, Bizerte) (Dam Ziatine, alt. 48m, 37°11'N, 9°11'E) Tunisia.

SAMPLING AND HOST PLANT INFESTATION LEVEL

Pupae collection was made in 2013 from mid-May to mid-June, on 30 trees of each plant species: *Q. coccifera*

(QC) and *P. lentiscus* (PL) in every station (Table 1). As a whole, 221 pupae were directly collected from the field. All were then placed individually in plastic boxes (8 cm height x 3cm Ø) at $25\pm 2^{\circ}\text{C}$ to follow mortality factors (dried pupae) and parasitism (parasitoid's emergence) (Table 1).

After two years of outbreak, in 2005 and 2009 (EZZINE *et al.*, 2015a), the pest remained at low level of infestation in Jebel Abderrahmane since 2012 (EZZINE, 2016). In Sejnane, the pest population remained at low level since it has been first recorded in 2010 inducing with the Gelechiidae *Anacamptis scintillella* the defoliation of trees and shrubs (including QC and PL) of this cork oak forest (EZZINE *et al.*, 2015b). In this work, host plant infestation was evaluated by counting the number of moth pupae on 30 trees of each plant species.

Table 1 – Number of pupae (N) collected from each station and on host plant species.

Station	Host plant	N
Ftahiz	<i>P. lentiscus</i>	68
	<i>Q. coccifera</i>	7
Delhiza	<i>P. lentiscus</i>	50
	<i>Q. coccifera</i>	38
Guitoun	<i>P. lentiscus</i>	26
	<i>Q. coccifera</i>	7
Dam Ziatine	<i>P. lentiscus</i>	8
	<i>Q. coccifera</i>	17

PARASITOID IDENTIFICATION

Emergent adults of parasitoids were preserved with ethanol (96%) in Eppendorf tubes (1.5 ml). Morphological identification was done at species level using keys: BOUČEK (1951) for *Brachymeria tibialis*, FITTON *et al.* (1988) for *Pimpla rufipes* and PECK *et al.* (1964) for *Monodontomerus minor*.

STATISTICAL ANALYSIS

Generalized linear models (GLMs) were applied to the following dependent variables: (1) number of pupae on each host species; (2) total dead pupae; (3) dried pupae and (4) parasitized pupae. Two explanatory categorical variables were tested: host species and stations. The best distribution model was chosen according to the deviance to the degrees of freedom (df) criterion. A Poisson distribution model best fitted the number of pupae on each host species and total of *M. minor* in each station. For the other variables, the Negative Binomial distribution model best fitted the data. Results are presented in the form of the Wald's chi-square test value (χ^2), parameter estimates and the respective P value.

RESULTS

HOST PLANT INFESTATION LEVEL

There is a significant difference of infestation level (mean number of pupae/tree) between the two host plants ($\chi^2_1 = 4.613$; $P=0.032$) but not between stations. The interaction term was significant ($\chi^2_2 = 7.623$; $P=0.022$). Number of pupae observed on PL was the highest in Ftahiz and the lowest in Dam Ziatine (Table 2) while, on QC the number of observed pupae was the highest in Delhiza and the lowest in Ftahiz and Guitoun (Table 2).

Table 2 – Average number ($\pm$ SE) of pupae in each station on both host plant species.

Station	Host plant	Number of pupae			
		mean/ tree	SE	min/ tree	max/ tree
Ftahiz	<i>P. lentiscus</i>	2.26	0.67	2	12
	<i>Q. coccifera</i>	0.23	0.1	1	2
Delhiza	<i>P. lentiscus</i>	1.66	0.48	2	10
	<i>Q. coccifera</i>	1.26	0.47	1	13
Guitoun	<i>P. lentiscus</i>	0.86	0.37	1	10
	<i>Q. coccifera</i>	0.23	0.12	1	3
Dam Ziatine	<i>P. lentiscus</i>	0.80	0.29	1	5
	<i>Q. coccifera</i>	1.1	0.3	1	6

GENERAL PUPAL MORTALITY

Among the 221 studied pupae, 165 adult moths emerged (74.6%). Pupal mortality was 25.3% as a whole. There was no significant difference between stations and host plants. In Ftahiz (42%) and Dam Ziatine (23%) general mortality rate was higher on QC (42% and 23% respectively) than on PL (26% and 13% respectively). Contrariwise, in Delhiza, mortality rate was higher on PL (38%) than on QC (16%). In Guitoun, mortality reached 15% on both host plants (Fig. I).

DRIED PUPAE

Statistical analysis showed that there is no significant difference of dried pupae between stations and host plants. In Ftahiz, dried pupae were observed only on PL (10%), while, in Delhiza and Dam Ziatine dried pupae were observed only on QC (24% and 16% respectively) (Fig. II). In Guitoun, percentage of dried pupae varied between 11% and 14% on both host plants.

PARASITISM

Parasitoid adults emerged from June 2013 to September 2013. No significant difference of parasitized pupae numbers was observed between host plants, but a highly significant difference was observed between stations ($\chi^2_3 = 14.654$, $P=0.002$). Parasitism was higher on QC in Ftahiz (43%) and Delhiza on PL (38%), whereas it

Fig. I – Global mortality rate of *O. trigotephra* pupae on *P. lentiscus* and *Q. coccifera*.

remained very low in Guitoun and Dam Ziatine (4% and 13% respectively) (Fig. II).

Three pupal parasitoid species belonging to the Hymenoptera order were identified (Fig. III): *Brachymeria tibialis* (Walker, 1834), *Pimpla rufipes* (Miller) and *Monodontomerus minor* (Ratzeburg).

Only one specimen of *B. tibialis* emerged in July 2013 from pupae collected on PL in Delhiza, also only one specimen of *P. rufipes* emerged from pupae collected on PL in Guitoun in June 2013 and, another one emerged from a pupa collected in Dam Ziatine in July 2013.

Monodontomerus minor was obtained from pupae collected on PL in both Delhiza and Ftahiz, without a significant difference of parasitism. Total number of *M. minor* reached 31 specimens in Delhiza and 10 in Ftahiz. Sex ratio was in favor of females in the two stations reaching 0.40 in Delhiza and 0.25 in Ftahiz.

Fig. II – Rate of Parasitism and dried pupae of *O. trigotephras* on *P. lentiscus* and *Q. coccifera* in each stations.

DISCUSSION

In our study, as a whole, population density of *O. trigotephras* was low (average density = 1.05 pupae/tree) and pupa mortality rather low (25%). We found that, except for Dam Ziatine (Sejnane) where the general infestation was the lowest, the number of pupae of *O. trigotephras* was higher on PL than on QC (Jbel Abderrahmane). EZZINE *et al.*, (2015a) showed that during outbreak years (2005 and 2009) in Jebel Abderrahmane, density of egg masses of *O. trigotephras* was higher on PL than on QC, while, at low population density (2014), infestation on QC was slightly higher than on PL. EZZINE *et al.*, (2015b) showed that in Sejnane and, during the 2010 outbreak, pupae of *O. trigotephras* were mainly observed on *Halimium halimifolium* (L.) Willk., *Q. coccifera* and *P. lentiscus*. However, larval parasitism was also higher on QC than on PL (HAMMAMI *et al.*, 2017) which can partly explain the inverse results we obtained for pupal numbers.

Host use for oviposition by *O. trigotephras* is ruled by resources exploitation in function of density dependent intraspecific competition (EZZINE *et al.*, 2015a) and interspecific competition between *O. trigotephras* and *Acrobasis consociella* (Pyrilidae) which also feed on QC (EZZINE *et al.*, 2016). Our study (2013) coincides with the retrogradation phase of *O. trigotephras* population in all stations. The general mortality varied between stations but not significantly and was higher on PL than on QC in Delhiza. This variability may be due not only to the difference in the density of QC trees that was higher in Delhiza than in the other stations (EZZINE, 2016) but also to the effect of larval parasitoids (HAMMAMI *et al.*, 2017).

Natural mortality (dried pupae) rate may be determined by the pest gradation level, the quantity and the quality of available food that mature larvae need to pupate. Likewise, competition between *O. trigotephras* and *A. consociella* was higher in Delhiza than in the other stations (Ezzine, *pers. obs.*) since neonate larvae feed on QC (EZZINE *et al.*, 2015a).

FRAVAL (1984) showed that the quality and the quantity

Fig. III – A. *Pimpla rufipes*: A1. Adult, A2. parasitized pupa. B. *Brachymeria tibialis*: B1. Adult, B2. parasitized pupa. C1. Parasitized pupa of *M. minor*; D1. Adult male of *M. minor*; D2. adult female of *M. minor*.

of food are among the most important factors in the regulation of *L. dispar* populations. HÉRARD (1984) showed that the old leaves of cork oak that persist are scorned as long as the new foliage is not exhausted, their consumption by the larvae of *L. dispar* causes high mortality and reduces the reproductive potential of the population.

General mortality caused by parasitism was low (5%). The pupal parasitism rate was significantly different between host species but not between stations. This may be due to the interaction between plant species. In fact, GRIPENBERG *et al.* (2010) showed that host plant selection and herbivore performance are correlated. Furthermore, CONNELL (1983) indicated that competition among different plant species includes all the limiting effects between plants (resources and allopathy) which reduce or prevent the growth and the survival of the plant.

Thus, the use of plants like PL that do not offer good food quality can be offset by an indirect positive effect "the escape from natural enemies" (EZZINE *et al.*, 2015a). It should be the case of *M. minor* that was observed only on PL in Jebel Abderrahmane. Forty-one specimens emerged in the lab from pupae collected on PL from early June to the end of September 2013. This Tormidae is a gregarious parasitoid of lepidopteran pupae and often a hyperparasitoid of Hymenoptera and Diptera via their lepidopteran and Hymenopteran hosts (STEFFAN, 1952; KULMAN, 1965; IWATA & TACHIKAWA, 1966).

The other parasitoids collected are too few to draw the same hypothesis. Only one specimen of *B. tibialis* was observed on PL in only one station (Delhiza) where it was already observed during an outbreak of *O. trigotephra*s in 2005 (Ezzine *pers. obs.*). *Brachymeria tibialis* is known to attack *L. dispar* during outbreak (VILLEMANT, 1989) in most European and North African countries.

Another common parasitoid of moths in Europe, *P. rufipes*, attacks a wide range of Lepidoptera species (VILLEMANT & FRAVAL, 1991), notably pupae of *L. dispar* and *O. trigotephra*s (DAJOZ, 2010). In our study (2013) two specimens of this ichneumonid emerged from pupae collected on PL in Guitoun and Dam Ziatine, while in 2009, it was observed on QC in December in Delhiza and in June in Dam Ziatine (Ezzine *pers. observ.*).

These results highlight the complexity of mechanisms of pupa mortality involving the relation between their host plant use and parasitoids (higher trophic level) and the difficulty to highlight them at low pest population densities. The assembly of parasitoids in a particular habitat depends indeed on host and environmental factors and may result in different degrees of natural control of a given pest (MILLS, 1994).

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