Bio-ecological study of hyperparasitoid aphids in the region of Mostaganem (north-western Algeria)

Amine Ghelamallah¹, Djilali Benabdelmoumene², Rachid Bouhraoua³, Malika Boualem¹, Mohamed Arbaoui¹, Mar FerrerSuay⁴ & Juli Pujade-Villar⁵

¹University of Mostaganem, Laboratory of Plant Protection, Department of Agronomy, Faculty of Nature Sciences, Algeria; e-mail: amine.ghelamallah@univ-mosta.dz; boualemmalika@yahoo.fr; medarbaoui58@gmail.com

²Université of Mostaganem, Applied Animal Physiology Laboratory, Department of Agronomy, Faculty of Nature Sciences Algeria; e-mail: benabdelmoumenedjilali@hotmail.com

³University of Tlemcen, Faculty of Nature Sciences, Forestry Department, Algeria, e-mail: rtbouhraoua@yahoo.fr

⁴Universitat de València, Facultat de Ciències Biològiques, Departament de Zoologia. Campus de Burjassot-Paterna, Dr. Moliner 50, 46100 Burjassot (València), Spain; e-mail: mar.ferrer.suay@gmail.com

⁵Departament de Biologia Animal, Facultat de Biologia, Universitat de Barcelona, Avda. Diagonal, 645, 08028 Barcelona, Spain; e-mail: jpujade@ub.edu

*Corresponding author, email: amine.ghelamallah@univ-mosta.dz

ABSTRACT Several species of hyperparasitoids belonging to the subfamilies Charipinae (Hymenoptera: Figitidae) and Pteromalinae (Hymenoptera Pteromalidae) have been identified in the region of Mostaganem (north-western Algeria). Among these species, two of them show a regular presence during the years of study, *Pachyneuron aphidis* (Bouche, 1834) (Pteromalidae), and *Phaenoglyphus villosa* (Hartig, 1841) (Figitidae). These two species have been proved to be effective in adapting very quickly to the environment.

KEY WORDS Aphids; Algeria, Mostaganem; Charipinae; Pteromalidae.

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INTRODUCTION

Hyperparasitoids are insects that evolve at the expense of primary parasitoids (Sullivan & Völkl, 1999). Within an ecosystem, they occupy the fourth trophic level (Buitenhuis, 2004). The vast majority of hyperparasitoids are hymenoptera, only a few species belong to Diptera and Coleoptera (Gordh, 1981, Sullivan, 1987). They are characterized being small (1 to 2 mm) but robust. Usually, they have a metallic coloration and their antennas have between 8 to 13 antennal segments. Their heads vary from sub-rectangular to oval. Hyperparasitoids are mostly generalists and not likely to depend on the presence of a specific parasitoid (Sullivan & Völkl, 1999). They can act as koïnobionts, meaning that

the female does not kill the host parasitoid at the time of laying the egg and idiobionts. The primary parasitoid is paralyzed or killed during the laying. For example, *Alloxysta* Foerster, 1869 is koïnobiont, while *Asaphes* Walker, 1834 is idiobiont (Sullivan, 1987).

Aphid hyperparasitoids can be classified into two categories according to their mode of development (Stary, 1970; Sullivan, 1987). There are endo-parasitoids and ecto-parasitoids. In the first category, the female lays the egg inside the primary parasitoid, while the aphid continues to live there. Once the aphid is ousted by the primary parasitoid and the mummy is formed, the egg of the hyper-parasitoid hatches and its larva feeds from the primary parasitoid pupa. In this group, there are hyperparasitoids belonging to the genus Alloxysta (Sullivan, 1987). The second category includes ecto-hyperparasitoids. Two elements distinguish the latter from the first group: the female attacks the mummy, which she pierces with her ovipositor and deposits an egg in the larva of the developing primary parasitoid. The egg hatches and the secondary larva feeds on the tissues of the primary parasitoid. This is the case of hyperparasitoids of the genus Asaphes, Dendrocerus Ratzeburg, 1852 and Coruna Walker, 1833 (Sullivan, 1987). The life cycle of hyperparasitoids is often related to the nutritional quality of the host. Most hyperparasitoids attack their host before mummification of the aphid for maximum benefit (Buitenhuis, 2004). These species attack either the larva of the parasitoid in the aphid before it is killed, or the parasitoid pupa or pupa in the aphid mummy (Buitenhuis, 2004).

In this study, the approach is based on a thorough knowledge of the population dynamics of the hyperparasitoid fauna, on the knowledge of the bioecological parameters intervening in the regulation of the hyperparsitoid populations, with an assessment of the impact of different abiotic factors (temperature in this case) on the biotic regulation of hyperparsitoid populations

MATERIAL AND METHODS

In this study the hyperparasitoid aphid has been found in Mostaganem provinces, north-western Algeria. These regions were chosen due to their important agricultural production in the country by the experimental exploitation of the Department of Agricultural Science at the Mostaganem University.

All the materials have been collected by the first author. The sampling unit used in this experiment is pepper (*Piper nigrum* L.).

The surveys were carried out once a week from January until July of the years from 2011 to 2014. The mummified aphids were separated and placed in microtubes and labeled Petri dishes up to the emergence of adult hyperparasitoids. The adults were identified by two of the authors specialists, Pujade-Villar and Ferrer-Suay (University of Barcelona, Spain).

The rate of hyperparasitism corresponds to the ratio number of hyperparasitoids counted on the

total number of parasitoids emerged (primary and secondary) times 100.

RESULTS

Of the two groups of hyperparasitoids recorded in this study, one belongs to family Figitidae (Cynipoidea). Here, we distinguish in particular the subfamily of Charipinae. Its members are widely distributed in all biogeographical regions (Ferrer-Suay et al., 2015). They are divided among 8 genera, namely: Alloxysta Forster, 1869 (cosmopolitan) Phaenoglyphis Forster, 1869 (cosmopolitan) Lytoxysta Kieffer, 1909 (North America), Lobopterocharips Paretas-Martinez & Pujade-Villar, 2007 (Nepal), Dilyta Forster, 1869, Apocharips Fergusson, 1986 (East Palearctic and the Neotropics), Dilapothor Paretas-Martinez & Pujade-Villar, 2006 (Australia) and Thoreauana Girault, 1930 (Australia). Alloxysta and Phaenoglyphisare are known as hyperparasitoids of aphids via Aphidiinae (Ichneumonoidea: Braconidae) and Aphelininae (Chalcidoidea: Aphelinidae) (Rabasse & Dedryver, 1983; Menke & Evenhuis, 1991).

The results of specimen identifications show hyper parasitoids that live at the expense of primary aphid parasites attacking several cultivated and herbaceous plants in western Algeria (Ferrer-Suay et al., 2015). Hymenoptera species belonging to Charipinae and Pteromalidae found in the study area are either secondary parasitoids or hyperparasitoids (Stary, 1970; Muller et al., 1999).

We recorded the presence of hyperparasitoids from March of every year of this study. Their manifestation has progressed over time. This evolution reaches the maximum rate during the month of May for two successive years (2012 and 2013) (Fig. 1). However, during the last year of observation (2014), we noticed a rapid and early increase in hyperparasitoids beginning in April. These rates of hyperparasitism were higher than in previous years. From May onwards, the rate of hyperparasitism decreases to a very low rate during the month of June, which vanishes towards the beginning of July (Fig. 1).

Under experimental conditions, aphid hyperparasitoids may live longer than 2 months depending on species, with a shorter longevity of males than females. In the laboratory, depending on the



Figure 1. Parasitism rate of parasitoids by hyperparasitoids during the study period (2012/2014).



Figure 2. Representation of hyperparasitism in the plan of A.F.C between 2012/2014 (annual effect).



Figure 3. Monthly representation of hyperparasitism in the plan of A.F.C between 2012/2014 (monthly effect).

species, the aphid mummifies about 8 days after the emergence of the primary parasitoid. Two and a half to three days after the mummification of the aphid, the larva of the hyperparasitoid begins to devour the primary parasitoid from which it eventually emerges. It will continue to feed on its remains and eventually emerge from the mummy a few days later. Depending on the species, the complete cycle of development of *Alloxysta* lasts between 13 and 20 days. The primary parasitoid stops its development and is transformed into a soft, blackish mass which serves as food for the *Asaphes* larva. About 21 days later, the adult emerges from the mummy (Table 1).

When we compare these results with those described in the literature, it appears that the results are equivalent to or less than those of other studies (Sullivan, 1987; Schooler et al., 1996; Buitenhuis, 2004). The duration of life cycle development is related to climatic conditions (Sullivan, 1987; Buitenhuis, 2004).

Factor analysis of correspondence (AFC) rates of hyperparasitism during 2012/2014

The factor analysis of the correspondences reveals that the peaks of hyperparasitism are recorded during the month of May 2012. However, it is important to note that the rate of manifestation of hyperparasitism recorded during the month of April 2013 is comparable to that of May 2012 (Fig. 2).

The analysis also revealed that the levels of hyper-parasitism recorded maximum values during the months of March, April and May (Fig. 3).

According to our analysis, the level of hyperparasitism is very important during the years of our study with a maximum reached in 2014 (Fig. 3). The same graphs show a close and positive relationship between spring and hyperparasitism. Nevertheless, the months of June and July 2013 have rates of hyperparasitoids lower than those of April and may be significant (Fig. 3).

If we compare the years of study with each other, we note that the rate of hyperparasitism differs from year to year. Indeed, during the years 2012 and 2014 the rates of hyperparasitism evolved, whereas in 2013 the rate of hyperparasitism remained very low. Indeed, the rate of hyperparasitism, for the years 2012 and 2014, exceeded 10%. This could be explained by the decrease in the abundance of parasitoids during this period. In 2013, the rate of hyperparasitism was only around 4%, which suggests that the action of biotic factors (presence of parasitoids) and abiotic factors (temperature and hygrometry) allowed the appearance of more and more hyperparasitoids.

Like the primary parasitoids, hyperparasitoids were unable to regulate host populations due to low parasitism and negligible effects on *Myzus persicae* populations (Buitenhuis, 2004).

Relative abundance of hyperparasitoids

During the study years, we recorded a relatively high relative abundance of hyperparasitoids. The species *Pachyneuron aphidis* (Bouche, 1834) (Pteromalidae Pteromalinae) is the most dominant with a proportion of 15%, followed by the species *Asaphes suspens* with a rate on the order of 10%. In the Figitidae Charipinae, *Phaenoglyphus villosa* (Hartig, 1841) represents a clear dominance in the harvested samples. In general, we found a higher abundance of Pteromalidae (58%) with a rate of 42% for Figitidae Charipinae (Fig. 4). This dominance can be explained by the adaptation to the climatic conditions of the medium by the Pteroma-



Figure 4. Average frequency of different families (sub-families) of hyperparasitoids during 2012/2014.

lidae (Hymenoptera Chalcidoidea) and Charipinae (Hymenoptera Cynipoidea Figitidae) (Fig. 4).

The results obtained during our work in the Mostaganem region on the abundance of hyperparasitoids confirm those obtained by previous works, such as Chehma (2013) in Ghardaïa (South Algeria) and Aggoun (2016), which cited several authors such as Halimi (2010) and Hemidi (2011) in Biskra (South-East Algeria) and Aggoun (2011) in Khenchela (East of Algeria), who consider that the biodiversity of hyperparasitoids studied in 2013/2014 reflects the importance of Pteromalidae in this region.

According to Sureshan & Narendran (2003), Pteromalidae is one of the most difficult group of study of the hyperparasitoids. It counts around 3400 species, distributed in 587 genera. Pteromalidae are natural enemies of several insect pests such as Coleoptera, Diptera, Lepidoptera, Hymenoptera and Hemiptera (Ghafouri-Moghaddam et al., 2014). Aggoun (2016) observed that hyperparasitoids in this family are the most active. Sureshan & Narendran (2003) were able to establish 15 tetra-trophic associations followed by species of the genus Asaphes. Moreover, it was found that the primary parasitoids belonging to the genera Praon Haliday, 1833 and Aphidius Nees, 1811 are the most affected by hyperparasitism. Pteromalidae play an important role in most ecosystems, mainly as secondary or tertiary consumers (Mitroiu et al., 2011).

In this study we analize the total parasitoids obtained on several corps in the region of Mostaganem (Algeria) (Table 2).

DISCUSSION

The inventory that we carried out during the period of study allowed us to identify several species of hyperparasitoids belonging to Charipinae (Hymenoptera, Cynipoidea, Figitidae) and Pteromalidae (Hymenoptera Chalcidoidea). Among these species, we mention two species which showed a regular presence during the years of study, *Pachyneuron aphidis* of the family Pteromalidae, and *Phaenoglyphus villosa* of the subfamily Charipinae. These two species have proved to be effective in adapting very quickly to the environment.

	Pre-imaginary levels	Longevity	Complete Biological Cycle
Charipinae	12±4.57js (b)	6.76±0.2js (b)	18.76± 6.57js (b)
Pteromalidae	14±2.74js (a)	8.72 ± 4.12js (a)	22.72±6.86js (a)
Average	13±3.65js	7.74±3.06js	20.74±6.71js

Table 1. Duration of biological development of hyper-parasitoid at 25 $^{\circ}$ C \pm 02 (days).

Aphid hyperparasitoids are those attracted only by aphids that have already been parasitized by a primary parasitoid (Sullivan, 1987; Sullivan &Völkl, 1999). In the absence of primary parasitoids, some hyperparasitoids transform into primary parasitoids and even occur on non-parasitized aphids (Sullivan, 1987; Sullivan & Völkl, 1999).

High parasitism is a primary parasitoid mortality factor that reflects the nature and extent of interspecific interactions between these two groups of insects (Chehma, 2013). It has been shown that when hyperparasitoids are present, females of primary parasitoids abandon their host plots without fully exploiting the resource in order to minimize the risk of mortality of their offspring (Chehma, 2013).

Unlike parasitoids, hyperparasitoids showed a steady and significant presence during the months of May and June. This presence is related to the decrease of the parasitoids during these two months when the temperature is unfavorable for their development. On the contrary, the hyperparasitoids showed a fairly high activity during the summer period.

In parallel, other authors reported significant activity during the summer. In Algeria at Ghardaïa, Chehma (2013) reported that the action of these hyperparasitoids became very prominent during the summer period, despite the micro-climatic conditions within the oases.

In Greece, Kavallieratos et al. (2005) report that the action of these hyperparasitoids becomes very prominent during the summer period. In Canada, Acheampong et al. (2012) note that rates of hyperparasitism were much higher in the greenhouse at the end of August and early September, at 77.78 and 77.38% respectively.

Family	Sub-family	Genre	Species
PTEROMALIDAE	ASAPHINAE	Asaphes	Asaphes suspensus (Nees, 1834)
	PTEROMALINAE	Pachyneuron	Pachyneuron aphidis (Bouché, 1834)
FIGITIDAE	CHARIPINAE	Phaenoglyphis	Phaenoglyphis villosa (Hartig, 1841)
		Alloxysta	Alloxysta arcuata (Kieffer, 1902)
			Alloxysta consobrina (Zetterstedt, 1838) = Alloxysta fuscicornis (Hartig, 1841)
			Alloxysta fracticornis (Thomson, 1862)
			Alloxysta pilipennis (Hartig, 1840)
			Alloxysta victrix (Westwood, 1833)

Table 2. Representation of hyper-parasitism in the plan of A.F.C between 2012/2014 (annual effect).

For example, all mummies of *Uroleucon sonchi* collected in June and July gave only hyperparasitoids (Willmerand & Unwin, 1981).

It has been demonstrated that when hyperparasitoids are present, primary parasitoid females abandon the plots of their phytophagous host without fully exploiting the resource in order to minimize the risk of their offspring mortality (Ayal & Green 1993, Hölleret al., 1994, Mackauer & Völkl, 1993, Weisser et al., 1994, Buitenhuis, 2004). They affect the effectiveness of primary aphid parasitoids by decreasing their abundance and modifying their behavior (Ferrer-Suay et al., 2014).

Our knowledge of the impact of hyperparasitism on the primary parasitoid of aphids is limited and highly fragmented (Buitenhuis, 2004). Rosenheim (1998) reports that hyperparasitism severely disrupts the short-term regulation of host populations by parasitoids. However, critical studies over several generations have yet to be conducted to assess long-term effects. Moreover, a precise knowledge of the natural history of certain important groups of hyperparasitoids is a prerequisite for improving our understanding of their origin, their distinctive biological attributes, and their role in the structure of communities (Brodeur, 2000).

This study aims a better understanding of biology and hyperparasitoid behavior as a contribution to unravel the nature of parasitoids - hyperparasitoids interactions.

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