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Ecological relationship between *Lepidosaphes beckii* (Newman, 1869) (Homoptera Diaspididae) and its two parasitoids *Aphytis melinus* DeBach, 1959 and *A. lepidosaphes* Compere, 1955 (Hymenoptera Aphelinidae) on lemons orchads in two localities of Mitidja Algeria

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ABSTRACT The study purpose is to understand the behavior of two hymenoptera parasitoids from *Aphytis* genus, *Aphytis melinus* DeBach, 1959 and *A. lepidosaphes* Compere, 1955, against armored scale host, *Lepidosaphes beckii* (Newman, 1869), in two lemon trees orchards of two localities: Heuraoua and Rouïba. The scale insect (*L. beckii*) develops 3 generations per year in Rouïba locality and 4 generations per year in Heuraoua locality. However, the parasitoid incidence of the two parasitoids species (*A. melinus* and *A. lepidosaphes*) are different between the two localities, according to the seasons and the plant organ. Indeed, the parasitism rate of *A. lepidosaphes* is higher in Rouïba than Heuraoua. Whereas, *A. melinus* activity is very important in Heuraoua and completely absent in Rouïba. Moreover, we noticed an important activity of *A. lepidosaphes* during the autumn and winter period, on branches while *A. melinus* is more active during summer period on scale populations attached to leaves. The comparative study of the morphometric measurements of *L. beckii* female parasitized covers between both localities demonstrate a positive relationship between the measurements of female covers and the parasitism rate recorded in these regions.

KEY WORDS *Aphytis lepidosaphes*; *Aphytis melinus*; *Lepidosaphes beckii*; Mitidja; morphometry.

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INTRODUCTION

Citrus are affected by many species harmful insects (scales). Armored scales are economically the most important, by damage and number (419 genus) (Amouroux, 2017). Several species have been recorded in the world and in Algeria. However, they are difficult to control because of their waxy cover protection (Balachowsky & Mensil, 1935). However, it is possible to break the life cycle of the insect by destroying young larvae, and using natural enemies (Coutin, 1988).

A large number of natural enemies are recorded in the *Citrus* ecosystem as predators and parasitoids that can decrease scales populations. Among parasitoid taxon, the species of *Aphytis* genus are primary ectoparasitoids and the most important natural biological agents of the scale insect (Rosen & Debach, 1974). Their effectiveness in biological control of scales has been successfully demonstrated since the 1970s, due to the intensive studies organized by the OILB group work (1974). According to Zappala et al. (2012), understanding the mechanisms of population differentiation in parasitoïds is essential. Indeed, many research works are directed towards this concept, citing the studies on interspecific competition between parasitoids undertaken by Sorribas & Garcia-Marı´ (2010), Sorribas et al. (2010), Cebolla (2018) which have shown the capacity of *Aphytis melinus* DeBach, 1959 to displace *A. crysomphalii* Mercet, 1912 on *Aonidiella aurantii* (Maskell, 1879). Unfortunately, in Algeria, very few studies have been done in details on the parasitoïds of armored scales until now (Moumen, 1989; Chergui, 1990; Moussaoui, 1990; Amr Chentir, 1990 and Biche & Bourahla, 1993). Except those carried out by Biche (1988) on the biology of *Aphytis maculicornis*, an external parasitoïd of *Parlatoria oleae* pest on olive tree.

In order to provide more knowledge needed in a biological control program, we conducted this study, which aims to better understand the two parasitoids distribution: *A. lepidosaphes* and *A. melinus*, towards their hosts, *Lepidosaphes beckii*. For this purpose, we carried out a comparative analysis of the development of the scale populations and its two parasitoïds on lemon trees between two different localities in Algeria (Heuraoua and Rouïba).

MATERIAL AND METHODS

The experiment was carried out in two lemon trees orchards (Eureka Lemon: *Citrus limon* var. *eureka*) located in two different regions: Rouïba (36°18'47.25''N) and Heuraoua (3°18'43.33''E), in the eastern part of the Mitidja, north of Algiers (Fig. 1).

In each locality, sampling were collected three times a month from October 2016 to December 2018. Each orchard was divided into 9 plots from each block, we choose one of the most infested trees. From four cardinal directions, as well as the center of each tree: 2 twigs of 20 cm long, 2 leaves and 1 fruit are taken. We examined 81 samples with a total of 14040 twigs, 14040 leaves and 7020 fruits.

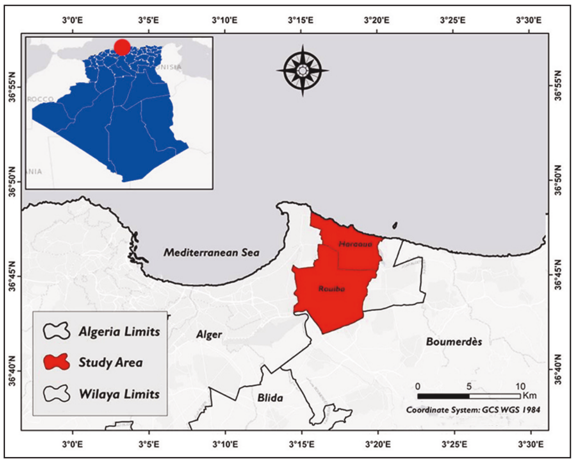

Figure 1. Location of the two studied orchards in the Mitidja plain (Algeria).

Samples were carefully observed under stereomicroscope to quantify the alive, dead and parasitized scales individuals and evaluate the impact of two Aphytis species (*Aphytis melinus* and *A. lepidosaphes*) on the scale (*L. beckii*).

Thus, the *Aphytis* parasitoids were identified according to their pupae coloration (Pekas, 2016). Besides, in order to understand the relationship between host size and parasitism, we measured the size of 50 parasitized covers of young females and 50 parasitized covers of adult females of *L. beckii* from each of the two localities in Algeria (Rouïba and Heuraoua), using a stereomicroscope with an ocular micrometer.

To calculate the surface of cover scale, we used a formula established by a Professor Leggat Réda, a mathematics teacher from the University of Boubaker Belkaid (Tlemcen-Algeria). This formula requires the identification of four micrometric dimensions (a, b, c, and d), from a triangular shape surface represented on a cover model of the female *L. beckii* (Fig. 2) under the following formula:

Parasitic cover surface in mm^2

$$\approx \left[\left(\frac{ac}{4}+\frac{bc}{4}\right)+\left(d*\sqrt{b^2+\frac{c^2}{4}}\right)\right]$$

Excel Stat 2014 software was used for the Statistical analyses. Our data are subjected to ANOVA, means were separated by Tukey's test at 5% level of significance.

RESULTS AND DISCUSSION

Scales recorded

In addition to the presence of *Lepidosaphes beckii* in both lemon trees orchards (Heuraoua and Rouïba), we also noticed other armored scales species such as: *Aonidiella aurantii*, *Chrysomphalus dictyospermi*, *Lepidosaphes gloverii*, *Parlatoria pergandii* and *Parlatoria ziziphi*.

The comparative study of the abundance rate between the different species of scale insects in the two localities showed that *L. beckii* and *A. aurantii* are the most abundant during the whole period of study, representing respectively a rate of 35.49% and 37.48% in the locality of Rouïba and a rate of 46.21% and 18.77% in the locality of Heuraoua (Fig. 3).

Parasitism incidence of A. melinus *and* A. lepidosaphes *on L. beckii in two localities*

The results of the comparative parasitism incidence in the populations of *L. beckii* between the two localities reveals that in Rouïba, *L. beckii* is only parasitized by *A. lepidosaphes* with 25.21% of parasitism rate. In Heuraoua, we found the presence of both parasitoids: *A. melinus* and *A. lepidosaphes*. However, *A. melinus* parasitized *L.beckii* with a rate of 27.12%, while its main parasitoid *A. lepidosaphes* showed a relatively low parasitism impact evaluated at 1.55%.

The parasitism rate induced by *A. lepidosaphes* in Rouïba locality (25.21%) is relatively high to that recorded in Heuraoua locality (1.55%) which remains very low (Fig. 4). While incidence parasitism caused by *A. melinus* is most important (27.12%). We expect that the presence of *A. melinus* and *A. lepidosaphes* in the same locality causes an interspecific trophic competition, affecting their parasitism incidence. Indeed, it appears that *A. melinus* was able to keep *A. lepidosaphes* partially away, which explains the low parasitism rate of this parasite.

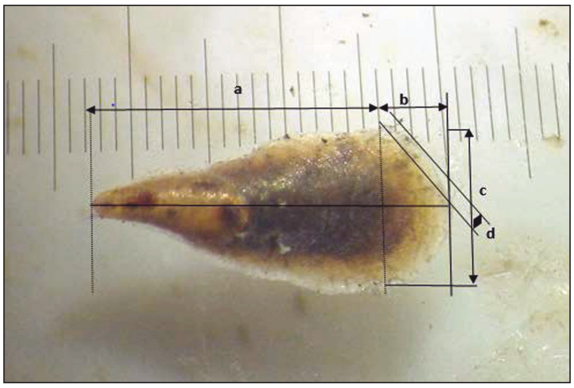

Figure 2. Identification of four micrometric dimensions (a, b, c, and d) from a cover area of *L. beckii*.

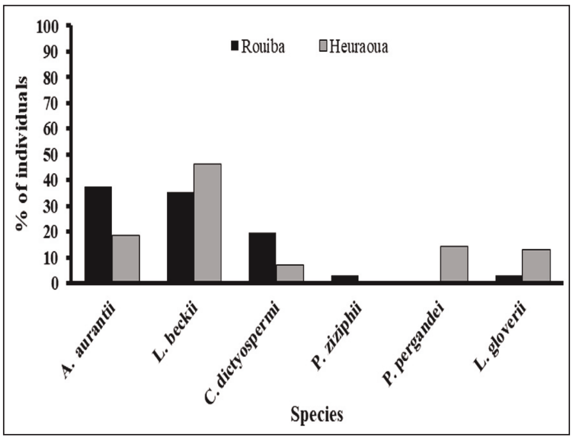

Figure 3. Importance of armored scales on lemon trees orchard in the two localities (Heuraoua and Rouïba) during the period 2016–2018.

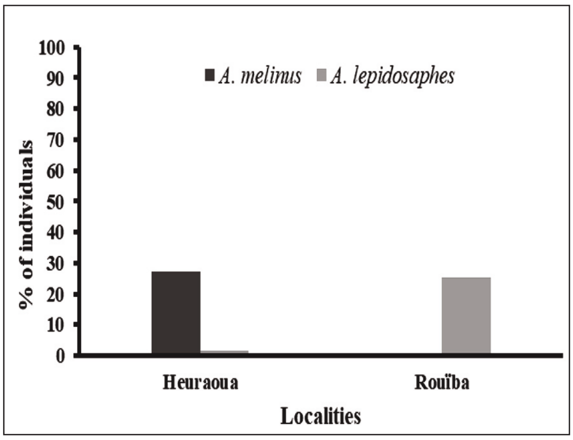

Figure 4. Parasitism incidence of *A. melinus* and *A. lepidosaphes* on *L. beckii* in the two lemon trees (Rouïba and Heuraoua).

Ecological behavior of the two parasitoids

The study of the comparative behavioral ecology of the two parasitoids on *L. beckii* in two localities (Rouïba and Heuraoua) allows us to discuss the interactions shown below.

Generation number and climate impact

From the Fig. 5, it appears that *L. beckii* showed 3 important periods of activity in the locality of Rouïba against 4 important peaks in the locality of Heuraoua during each of the two study years. We also found that the development of the two parasitoids follows the same evolution as their scale host.

The seasonal dynamics monitoring of scale-parasitoid complex in the two stations over two study years is mainly related to climate that characterized the two localities. Indeed, the results show that *L. beckii* develops 3 annual generations in Rouïba while 4 generations per year are recorded in Heuraoua.

This difference in the number of generations of the scale insect *L. beckii* is probably related to the microclimatic conditions of each area, especially the high relative humidity in Heuraoua compared to Rouïba. This is because of the orchard of Heuraoua is characterized by the effect of a relative humidity rate due to its location between the Mediterranean Sea and Lake Regaia. Our results are

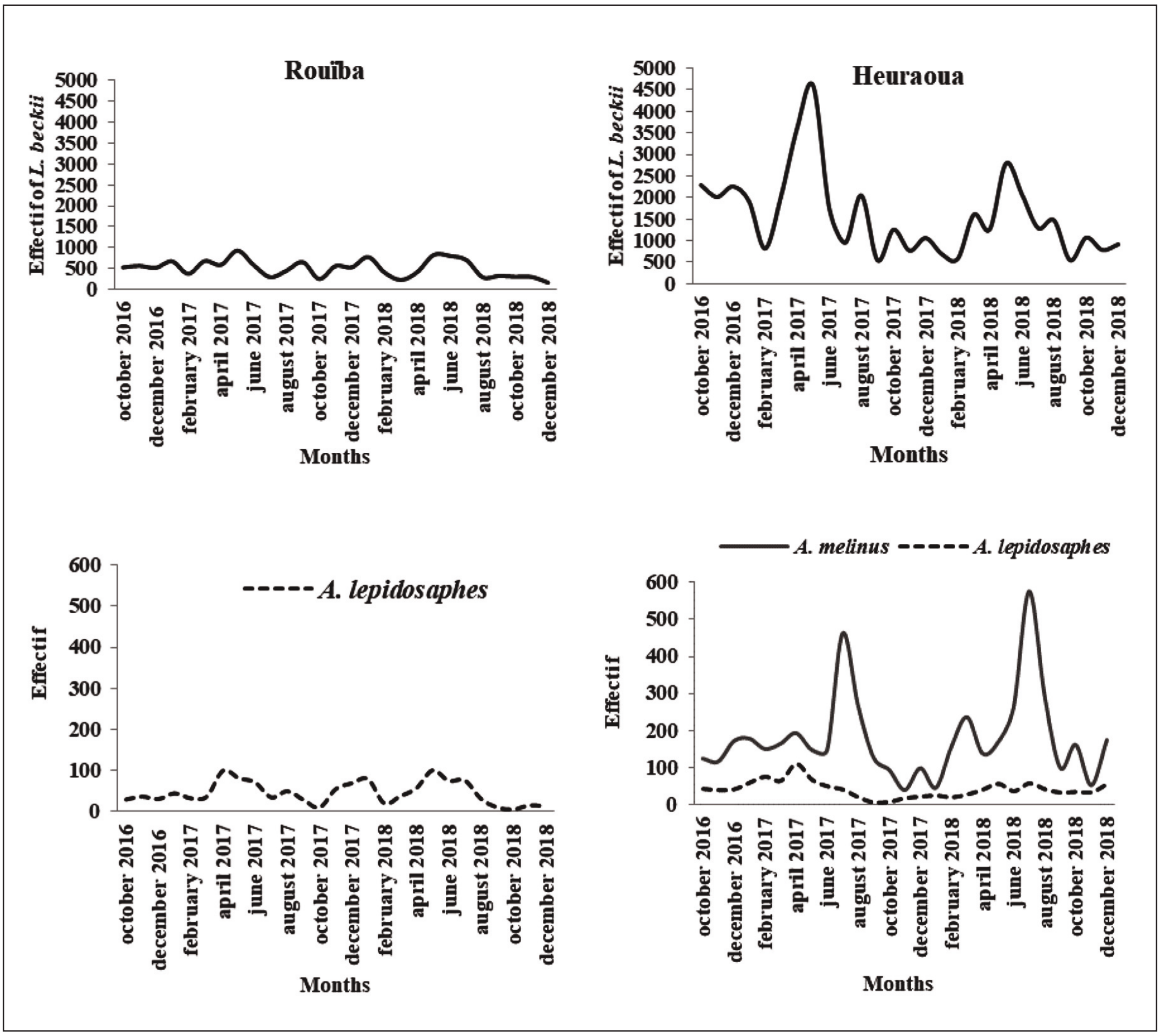

Figure 5. Fluctuation of *L. beckii* population and its two parasitoids (*A. melinus* and *A. lepidosaphes*) on two Lemon trees orchards (Rouïba and Heuraoua).

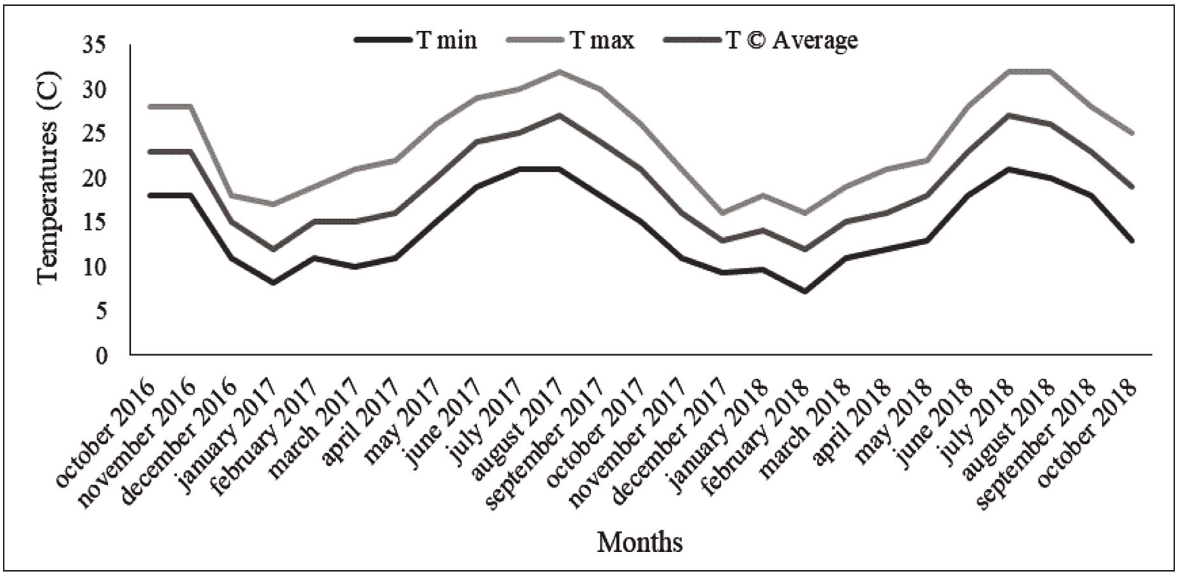

Figure 6. Temperature fluctuation in the two localities (Rouïba and Heuraoua) for the period: 2016/2017 and 2017/2018.

in agreement with Cohic work's (1955) who noticed that this scale has an affinity for shady places characterized by high humidity.

We also noticed that the development of both parasitoids is in perfect synchronization with the availability and development of their host *L. beckii*. From Kfir & Luck (1984), Duale (2005), Kalyebi et al. (2005), Peacock et al. (2006) and Sorribas et al. (2010), the parasitoids have a direct and close relationship with their hosts and share the same development conditions. However, the individuals number of both parasitoids is variable during the study period. In fact, *A. lepidosaphes* reached a maximum of its abundance during the period from October to February–autumn and winter period - during the two years of study. Then a progressive decrease is observed with the arrival of the great temperatures (July–August). It seems that *A. lepidosaphes* is better adapted to low temperatures (cold period). On the other hand, *A. melinus* populations reach their maximum abundance during the summer period (June to August) in both years of the study. We can say that *A. melinus* prefers high temperatures for its optimal development. This is confirmed by the maximum temperatures (≥ 30°C) recorded during the period from June to August (Fig. 6). The studies of Pekas et al. (2010) are in agreement with our results by reporting that this parasitoid shows a high frequency in localities characterized by hot periods.

The two parasitoids activity towards their host is related to several factors, including climatic factors (temperature and relative humidity) which have a major effect on the natural enemies development. The presence of an additional generation of the scale insect in the Heuraoua region is probably related to the high humidity level (close to 100%). Moreover, the low activity of *A. lepidosaphes* in the summer period can be explained by the high occurrence of *A. melinus* in this region. From Podoler et al. (1983), seasonal climatic variations in temperate zones allow an eco-subordinate parasitoid to coexist in the same locality with a dominant parasitoid when the latter is badly adapted in hot or cold periods.

Distribution according to the plant organs.

In the two localities (Rouïba and Heuraoua), *L. beckii* populations as well as those of the two parasitoids are differently distributed on the plant organs of the lemon trees (twigs, leaves and fruits). In effect, in the Rouïba region, we found that the *L. beckii* populations are more abundant on the twigs during the two years of study. The parasitism rate induced by *A. lepidosaphes* is high recording 58.31% for the first year (2016/2017) and with

31.66% during the second year (2017/2018). In Heuraoua locality, *L. beckii* is more active on the leaves especialy on the underside. Similarly, we noticed a high parasitism rate with 53.7% during the 1st period for *A. melinus* and 30.39% rate incidence for *A. lepidosaphes*. In the 2nd year, *A. melinus* showed the same parasitism incidence as in the previous study period (2016/2017) while for *A. lepidosaphes* the parasitism rate was 26.97% evaluated.

Parasitoids preferentially concentrate in areas where their scale hosts are at high densities (Wajnberg & Ris, 2007). The data collected on the spatial distribution of parasitoids show that these natural enemies are located where their host populations are important. They develop a parasitism pressure proportional to the scale density, which shows their great power of adaptation.

Oviposition behavior of the two parasitoids

During our study period, different observations were noticed concerning the oviposition behavior of the two parasitoids (*A. melinus* and *A. lepidosaphes*) towards their scale host (*L. beckii*) between the two localities (Rouïba and Heuraoua) (Fig. 8).

In fact, in Rouïba we have observed that the female of *A. lepidosaphes* lays often only one egg on its host but can sometimes lay up to two eggs on the adult female of the scale insect. In total, 101 eggs were counted throughout our study period. However, in Heuraoua, we noticed that *A. melinus* laid only one egg on the virgin female while on the adult female, characterized by a large size, 2 eggs per host are frequently counted. In total, 369 eggs are laid by *A. melinus* on *L. beckii* in this region. Concerning the female of *A. lepidosaphes*, we observed that it lays only one egg on the virgin female of *L. beckii*, with a total of 83 eggs during the entire study period.

According to the locality, we observe an intensive oviposition activity in Heuraoua than in Rouïba. This can be explained mainly by the favorable ecological conditions for the development of the two parasitoids in Heuraoua region. On the other part, it is noticed that the scale is parasitized only once, since *Aphytis* is able to detect beforehand the parasitized scale and leave the one already attacked (Abbassi, 1980). Ludeho (1968) reports that the development of *A. lepidosaphes* larvae on a virgin female of *L. beckii* often causes a deformation of the cover which allows Aphytis to recognize the parasitized forms externally (Fabres, 1974). On adult female scale, we counted two eggs of the same parasitoid species. Indeed, the female of *A. melinus* and *A. lepidosaphes* during the oviposition often deposited two eggs each on the body of their hosts

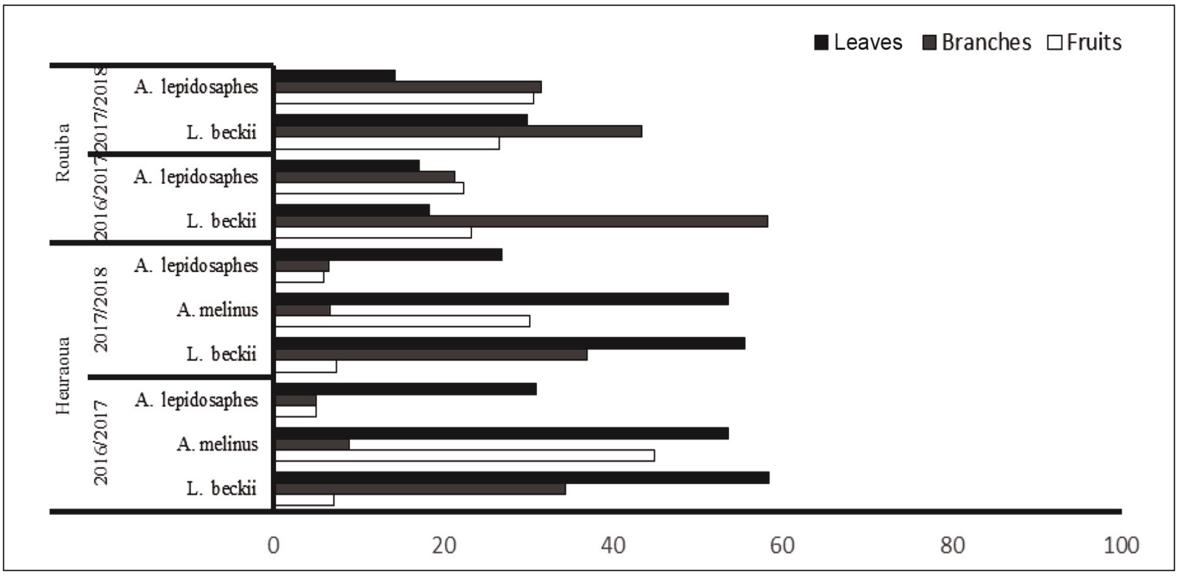

Figure 7. Distribution of *L. beckii* and its two parasitoids according to the plant organ on lemon trees in the two localities (Rouïba and Heuraoua) during 2016–2017 and 2017–2018.

(Gregarization phenomena). Nicholson & Bailey (1935) state that *A. lepidosaphes* is gregarious and specific species, it lays eggs only on *L. beckii*. In addition, Gonzalez (2015) reports observing the phenomena of facultative gregarism in *A. melinus*. On the other hand, we found that *A. melinus* completely dominates on adult females in the Heuraoua where *A. lepidosaphes* oviposition is absent on physiologically older hosts (adult female). This can be explained by the fact that *A. melinus* could compete and eliminate *A. lepidosaphes* from the adult female stage.

Indeed, *L. beckii* has physiological resistant individuals, which encourages species of the genus *Aphytis* to adopt a reproductive strategy that provides good development. However, it has been reported by Winkler (1981) and Briggs (1993) that the resistant host determined by a large size (adult stage), will be monopolized by the dominant parasitoid. In this case, it is *A.melinus* in Rouïba. Pekas et al (2016) add that this parasitoid always wins by occupying the best scale. However, the females of the secondary parasitoid will be obliged to parasitize a smaller scale host which exposes a competitive behavior between parasitoid species. Moreover, *A. lepidosaphes* only parasitizes virgin females of *L. beckii* in the Heuraoua locality, so it only competes with *A. melinus* on virgin females.

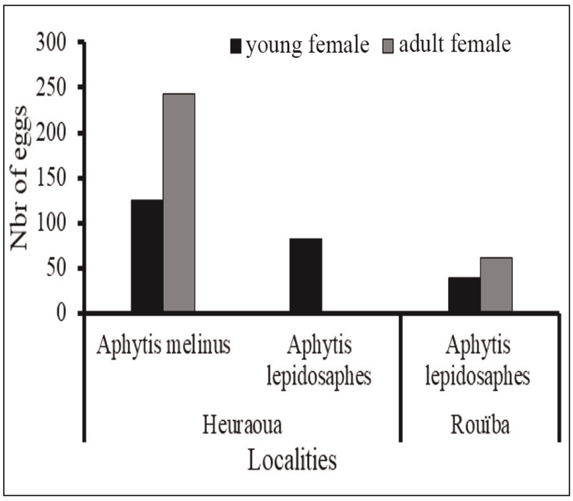

Figure 8. Number of eggs laid by the female of each of the two parasitoids (*A. melinus* and *A. lepidosaphes*) on female individuals (young female and adult female) of *L. beckii* in the two localities (Heuraoua and Rouiba).

Host	Parasitised cover size (Heuraoua)	Parasitised cover size (Rouïba)
Young female	9.61 a	7.09 b
Adult female	21.48 a	14.21 b

Table 1. Comparison of cover size of *L. beckii* females parasitized by *A. lepidosaphes* and *A.melinus* between the two localities (Heuraoua and Rouïba).

Measurement of the parasitized covers of females

In order to understand the behavior of the parasitoids against the same host, a comparative study of the measurements of the covers of the parasitized *L. beckii* females was carried out between the two localities (Rouïba and Heuraoua).

The size comparison of the parasitized covers between the two localities shows that those noticed in Heuraoua are larger than those in Rouïba locality. Indeed, statistical analysis reveals that for virgin females ($F = 30.48$; DF. = 99; $p < 0.0001$) as well as for adult females ($F = 63.92$; DF. = 99; $p < 0.0001$), individuals from Heuraoua are significantly larger than those from Rouïba. The Tukey test shows the existence of two groups (a and b) (Table 1).

The results obtained show that the parasitized covers of females from Heuraoua are larger than those from Rouïba. It may explain the important parasite activity observed in Heuraoua region where we noticed the presence of two parasitoids: *A. melinus* and *A. lepidosaphes* with a total of 452 parasitoid eggs counted. In addition, the presence of two eggs per host was often encountered in this locality. Whereas in Rouïba, only one parasitoid was noticed during our study, with a relatively low oviposition activity of about 101 eggs. Effectively, host size is known to have an important influence on various components of the physiological condition of adult *Aphytis*, such as longevity, fecundity, or searching ability of the host (Godfray, 1994). As a consequence, larger hosts are better quality as they should result in better and more efficient parasitoids (Pekas, 2010). This is in accord with our results and confirms the impact of host size on parasitism. Similar results are noticed by Boudjemaa et al (2020) who report the positive relationship between the host size of *Aonidiella aurantii* and the parasitism rate of *A. melinus*. From this, we can concluded that the high parasite activity in Heuraoua locality compared to Rouïba region is directly related to the host size.

On the other side, the scale size can be significantly affected by climatic variations in different localities (Pekas, 2010). However, the difference in the host size between the two localities may be related to the microclimate and host plant effect that characterized each of the two regions and that influence the host plant and indirectly the scale size. According to Fabres (1979), the influence of the perfect thermo hygrometric conditions of the habitat are very favorable to the optimal development of *L. beckii*. In this case, the orchard situated in the Heuraoua locality is exposed to a very high level of humidity (the sea and the lake approximately from the orchard) compared to the Rouïba orchard. We think that this high humidity level could favorite the presence of large scales in Heuraoua. On the other side, Biche & Sellami (1999) showed that the host plant acted as a real ecological factor where the action is translated on the duration of the cycle, the sex ratio, the fecundity and also on the females.

CONCLUSIONS

In this study, several species of scale insects were observed but with very variable abundance rates. However, the scale *L. beckii* and *A. aurantii* were the most abundant in the two localities (Rouïba and Heuraoua).

In addition, we noticed the presence of two parasitoids: *A. lepidosaphes* and *A. melinus* in the *L. beckii* populations. These two hymenoptera are capable to limitate effectively the population of purple scales in an eventual biological control program. Furthermore, we found that the development of *A. melinus* and *A. lepidosaphes* is closely related to their host scale evolution, climatic factors such as temperature and relative humidity, as well as to the spatial distribution and developmental stage of their host. However, we observed competition between the two parasitoids in the Heuraoua locality. In effect, *A. melinus* showed a superior parasitism action than *A. lepidosaphes*, the primary natural enemy of *L. beckii*. Moreover, comparison of the parasitized cover size of *L. beckii* females between the two localities revealed the presence of a positive correlation between the parasitized cover size of the parasitized scale and the parasitism rate.

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