

Morphometric comparison between two populations of *Aonidiella aurantii* (Maskell) (Homoptera: Diaspididae) from Algeria and Turkey

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The size of California red scale *Aonidiella aurantii* (Maskell, 1879) (Homoptera: Diaspididae) is the most reliable indicator in terms of host quality for *Aphytis melinus* (DeBach, 1959) (Hymenoptera: Aphelinidae) as well as for the efficiency of its biological control. Our study consisted in comparing the cover and body size of each scale developmental stage belonging to two different populations: one from Algeria and the other one from Turkey. The two scale populations were taken from lemon trees during three months. We compared measurements between the two localities and also between the plant organs. The larger individuals were those from Algeria. The same results were confirmed through the plant substrate on which scale was fixed: this size variation observed is mainly explained by climatic variations between the two countries and its repercussions on phenology and metabolism of the host plant. In addition, a higher parasitism rate was noticed in the Algerian scale population compared to that of Turkey.

Keywords: *Aonidiella aurantii*; size of individuals; Algeria; Turkey; *Aphytis melinus*

Introduction

Aonidiella aurantii (Maskell, 1879) (Homoptera: Diaspididae) is considered to be one of the most threatening pest of citrus worldwide (Pekas et al., 2016). However, given the difficulty in controlling it chemically, biological control is an excellent alternative. *Aphytis melinus* (DeBach, 1959) (Hymenoptera: Aphelinidae) is recognized as the most successful natural enemy against the California red scale (Forster & Luck, 1996) since, along with parasitism, it engages in the feeding of the host, causing a significant mortality (Tena et al., 2011). Nevertheless, the effectiveness of this ectoparasitoid is closely related to several factors that can limit its distribution and affect its abundance. Indeed, before making egg-laying decisions, the species of the genus *Aphytis* use a combination of physical characteristics of the host such as the size, the cover shape and the kairomone (Baker, 1976).

According to Hare & Morgan (2000), Hare & Luck (1994) and Hare et al. (1993), the concentration of the non-volatile compound O-caffeoyltyrosine, used by *A. melinus* as a kairomone for the recognition of the host, is qualitatively related to the scale body size. The notion of host size is particularly important for the majority of parasitic hymenoptera, as it can affect the physical condition by affecting longevity, fertility and research capacity (Godfray, 1994; Opp & Luck, 1986).

The host size plays a major role in the effectiveness of the parasite; however, *Aonidiella aurantii* can manifest different sizes depending on where it is found (Pekas et al., 2010; Luck & Podoler, 1985). Thus, a study on scale measurements could help to understand and improve the biological fight against this pest. It is in this context that this work took place, which aimed at comparing the scale size in two different localities, namely: Algeria and Turkey. In order to confirm our results, the comparison was carried out first without taking into account the plant organ and then with consideration of the plant substrate on which the scale was fixed. This work also aimed at highlighting the relation between the California red scale size and parasitism, which represented the main point of this study.

Materials and Methods

Two lemon orchards of the Eureka variety were selected mainly by the abundance of *Aonidiella aurantii* and the natural presence of *Aphytis melinus*. The first orchard was a private farm located in the region of Rouïba (East of Mitidja – Algeria, 36°43'49" N; 3°07'27"E); the second one was located in Batı Akdeniz Tarımsal Araştırma Enstitüsü (BATEM) in Antalya (Turkey, 36°55'22"N; 30°00'23"E). The two orchards were under the same management.

Samples were taken twice a month in september, october and november, 2018. At each field visit, we choose six most infested trees. From each tree, we took five leaves and two fruits from each cardinal direction, with a total of 150 leaves and 60 fruits per field visit. In laboratory, samples were first carefully examined under a binocular magnifier to determine the developmental stage and the state of each individual (parasitized or not). After that, the cover was separated from the body of the scale to carry out the measurements. On the other hand, the adult female stage where the cover and the body remain adhered, the measurements were carried out without separation. In addition, on females parasitized by *Aphytis melinus*, only the cover size was taken into account, because the body was partially or entirely consumed by the parasitoid. Measurements

were carried out by using a stereomicroscope with a micrometer in eyepiece. The calculation of the surface (in mm²) was performed by using the maximum length and the maximum width, whether for the cover or for the body of all scales individuals within 0.01 mm (Fig. 1). The data were analysed with ANOVA; the mean values were separated by the Tukey's test at a significance level of 5%.

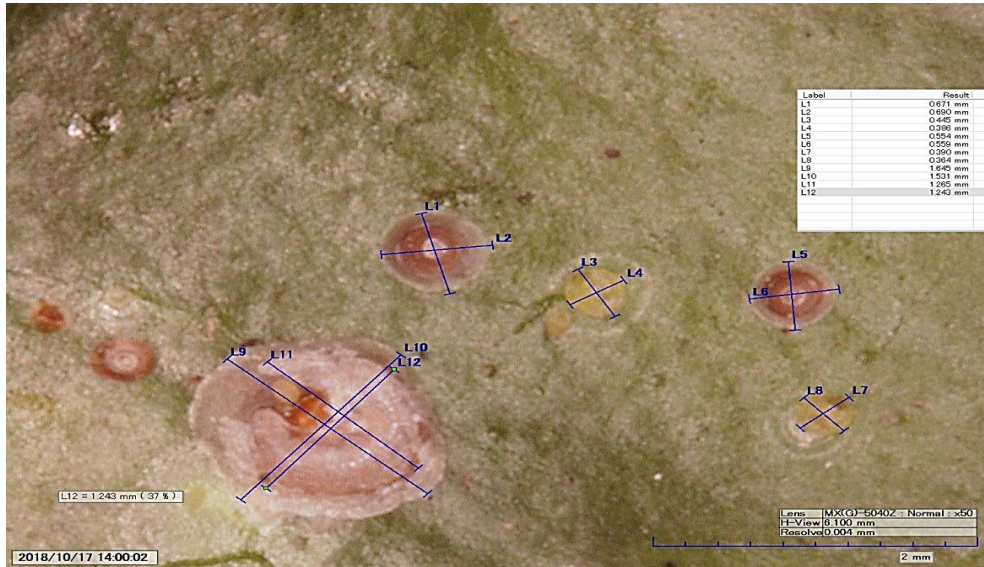


Fig. 1. Individuals of *A. aurantii* measured with stereomicroscope.

The processing of results was as follows:

Comparison of the scale size between the two localities: We compared the average (mean \pm SE) cover size (CS) and body size (BS) of all developmental stages of *A. aurantii* from Algeria (ALG) with that from Turkey (TRK). We also compared the temperatures between the two localities (Algiers and Antalya) during the study year (<http://www.Tutiempo.com>).

Comparison of the scale size according to the plant organ: We compared average measurements (mean \pm SE) of the covers and the bodies of physiologically older stages (nymph, virgin female, adult female) of the insect between the two countries (Algeria and Turkey) taking into account the plant organ (leaf and fruit) on which the scale was fixed.

Relationship between the scale size and the parasitism: We compared the average cover size of virgin females parasitized (CSP) by *A. melinus*, with that of virgin females no parasitized (CSN) and evaluated the parasitism rate on the two plant organs (leaves and fruits) in the two scale populations (Algerian and Turkish).

Results

Comparison of the scale size between the two localities

The results reported in Table 1 show that larger scales are those of Algeria compared to those of Turkey.

Table 1. The average size (mm²) of cover (CS) and body (BS) of different *A. aurantii* developmental stages according to the locality (Algeria and Turkey). For each parameter (CS and BS) the row means with different letters differ significantly ($p < 0.05$).

Developmental stages	CS (ALG)	CS (TRK)	BS (ALG)	BS (TRK)
1 st fixed stage	0.17 \pm 0.001 a	0.16 \pm 0.001 a	0.12 \pm 0.001 a	0.11 \pm 0.001 a
2 nd stage male	0.40 \pm 0.002 a	0.38 \pm 0.002 b	0.21 \pm 0.003 a	0.18 \pm 0.002 b
2 nd stage female	0.47 \pm 0.006 a	0.37 \pm 0.006 b	0.32 \pm 0.003 a	0.28 \pm 0.004 b
Prepupa	0.51 \pm 0.006 a	0.46 \pm 0.004 b	0.23 \pm 0.003 a	0.19 \pm 0.005 b
Pupa	0.71 \pm 0.001 a	0.58 \pm 0.002 b	0.28 \pm 0.001 a	0.22 \pm 0.002 b
Virgin female	0.95 \pm 0.002 a	0.71 \pm 0.002 b	0.69 \pm 0.002 a	0.50 \pm 0.002 b
Gravid female	2.03 \pm 0.004 a	1.83 \pm 0.002 b	1.63 \pm 0.004 a	1.43 \pm 0.003 b

Statistical analysis did not show any significant difference in size for the first stage between the two scale populations (Algerian and Turkish) (Cover: $F=3.60$; $d.f.=1, 43$; $P=0.064$. Body: $F=2.90$; $d.f.=1, 43$; $P=0.096$). On the other hand, we noted a significant difference between the two scale populations of Algeria and Turkey for the physiologically younger stages: 2nd stage female (Cover: $F=18.15$; $d.f.=1, 11$; $P=0.002$. Body: $F=13.41$; $d.f.=1, 11$; $P=0.004$), 2nd stage male (Cover: $F=9.61$; $d.f.=1, 11$; $P=0.011$ Body: $F=6.20$; $d.f.=1, 11$; $P=0.031$) and prepupa (Cover: $F=7.77$; $d.f.=1, 11$; $P=0.019$ Body: $F=8$; $d.f.=1, 11$; $P=0.017$). As for the physiologically older stages, Anova analysis showed a highly significant difference between the two scale populations (Algerian and Turkish): pupa (Cover: $F=200.90$; $d.f.=1, 79$; $P<0.0001$. Body: $F=28.25$; $d.f.=1, 79$; $P<0.0001$), virgin female (Cover: $F=107.57$, $df=1.111$, $P<0.0001$. Body: $F=65.79$, $df=1.111$, $P<0.0001$) and adult female (Cover: $F=35.23$, $df=1.127$, $P<0.0001$. Body: $F=28.31$, $df=1.127$, $P<0.0001$). Indeed, Algerian scale individuals were larger than those of Turkey, confirmed by the cover and body size.

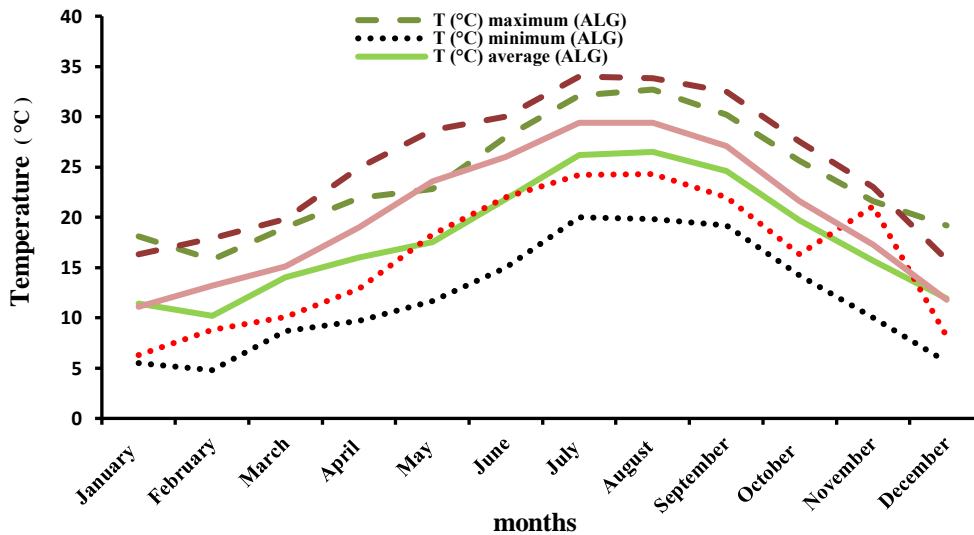


Fig. 2. Temperature fluctuation in Algiers and Antalya during 2018.

Comparison of the scale size according to the plant organ

The size comparison of each *A. aurantii* stage between the two populations fixed on the same plant organ (Table 2) indicated that, for the two parameters (CS and BS), Algerian scale population always remained larger than Turkish scale population.

Table 2. The average size (mm²) of cover (CS) and body (BS) of physiologically older stages (pupa, virgin female and adult female) according to leaves and fruits) and locality (Algeria, ALG and Turkey, TRK).

Developmental Stages	<i>Aonidiella aurantii</i> fixed on leaf				<i>Aonidiella aurantii</i> fixed on fruit			
	CS (ALG)	CS (TRK)	BS (ALG)	BS (TRK)	CS (ALG)	CS (TRK)	BS (ALG)	BS (TRK)
Pupa	0.69±0.003a	0.57±0.001 b	0.25±0.002a	0.21±0.001b	0.73±0.002a	0.58±0.002b	0.30±0.002a	0.24±0.002b
Virgin female	0.89±0.005a	0.67±0.004 b	0.62±0.004a	0.44±0.004 b	1.01±0.003 a	0.75±0.003b	0.76±0.004a	0.56±0.003b
Gravid female	1.96±0.007a	1.78±0.003 b	1.57±0.006a	1.37±0.005b	2.10±0.008 a	1.87±0.003b	1.70±0.008a	1.49±0.006b

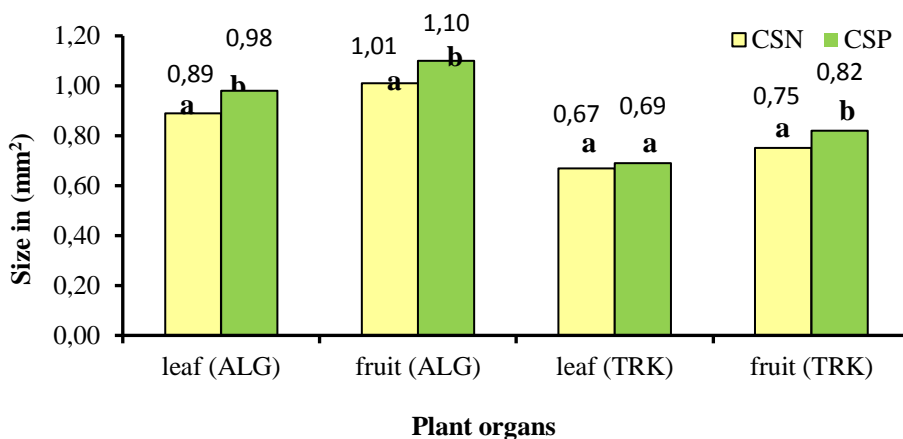


Fig. 3. Comparison between the average size of parasitized (CSP) and non-parasitized covers (CSN) according to the plant leaves and fruits in two populations (Algeria and Turkey). Means designated with different letters differed at p < 0.05.

Statistical analysis revealed that Algerian scale individuals remained significantly larger than Turkish individuals, regardless of the plant organ on which they are attached. On leaf: pupa (Cover: F=26,15; d.f.=1, 39; P < 0.0001. Body: F=14.07; d.f.=1, 39 ; P=0.0005), virgin female (Cover: F=41.99; d.f.=1, 55; P < 0.0001. Body: F=37.85; d.f.=1, 55; P < 0.0001) and adult female (Cover: F=15.09, df=1.63, P=0.0002. Body: F=16.78, df= 1.63, P=0.0001) also on fruit: pupa (Cover: F=127.44, df=1.39, P < 0.0001. Body: F=23.22; df=1.39, P < 0.0001), virgin female (Cover: F=112.94, df=1.55, P < 0.0001. Body: F=53.63, df=1.55, P < 0.0001) and adult female (Cover: F=23.93, df=1.63, P < 0.0001. Body: F=14.41, df=1.63, P=0.0003). For two populations and for the two parameters (CS and BS), plant substrate did not act on *A. aurantii* size variation according to the locality and the largest scale sizes were recorded on leaves and fruits of Algeria.

Relationship between scale size and parasitism

The average size of parasitized cover (CSP) was larger than the average size of non-parasitized cover (CSN) on the two substrates and in the two insect populations (Ffig. 3). The statistical results reveal that *A. melinus* highly parasitized covers having the largest sizes present on fruits for Algerian scale population (F=8.65; df=1.43, P=0.0005) and significantly covers of

large sizes found on leaves in Algeria ($F=4.76$, $df=1.38$, $P=0.03$) and on fruits in Turkey ($F=5.18$, $df=1.35$, $P=0.029$), while scales attached to leaves in Turkey, large sizes were not significantly parasitized ($F=0.06$, $df=1.39$, $P=0.793$). The parasitism level was higher in Algerian scale population compared to the Turkey population, but within the same population (Algerian or Turkish), the parasitism rate was higher on fruits than on leaves (Fig. 4).

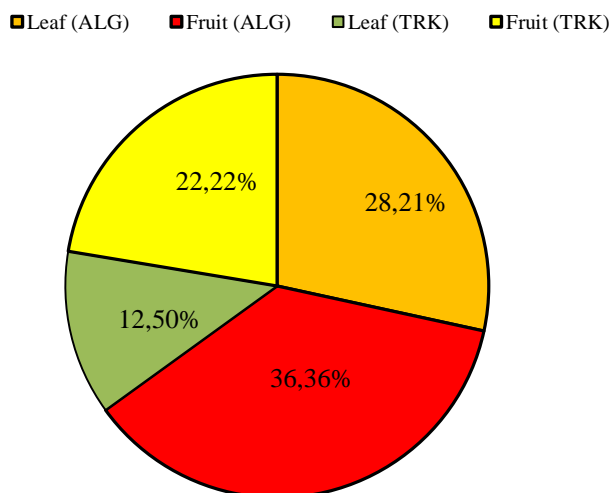


Fig. 4. Parasitism rate according to the plant organ and the locality of *A. aurantii*. For each plant organ (leaf and fruit) and each country (Algeria and Turkey).

Discussion

From the results of the size comparison between the two countries, it seems that geographic variation has a significant influence on scale size and it's considered to be an important agent influencing the scale size. Indeed, (Luck & Podoler, 1985) noted significant differences between localities of citrus orchards in California. They found that covers were smaller going from the coast towards the interior of California, with a tendency related to the climate in the scale size variation. On the other hand, Citrus fruits thrive well where the climatic conditions are favorable, according to researcher (Loussert, 1985) an optimum of vegetation oscillating between 22 and 26 °C whereas beyond 30 °C we noted a stop tree vegetation. In this case, maximum temperatures above 32 °C are recorded in July, August and September in Antalya coincide with the summer sap thrust (second sap growth) which certainly causes a slowing or stopping of the sap thrust, supposed to provide essential elements for the development of the lemon tree and indirectly that for the scale among others potassium, which affects the primary metabolites in plant tissues and plays important roles in animal cells life.

So, we think that the scale size variation between the two countries would therefore be related not only to climatic conditions but also to the chemical and biochemical constituents of lemon trees derived from the quality and quantity of sap thrust. Indeed, in a study on *Parlatoria oleae* (Colvée, 1880), Biche & Sellami (1999) affirmed that the host plant intervenes as a true ecological factor whose action is superimposed on other factors. In addition, a reduction of about 50% in the body size of adult females of California red scale is observed under seasonal influences in Spain (Pekas, 2011). Other works have shown that a notable seasonal variation on scale size, which they explained by the effect of temperature (Hare & Morgan, 2000; Hare & Luck, 1994; Hare et al., 1990; Yu & Luck, 1988).

Concerning the comparison of the scale size between the two localities according to the plant organ, in Algeria *A. aurantii* showed a marked preference for fruits compared to other parts of the tree (Biche et al., 2012). In Turkey, the scale was more abundant on fruits than on twigs and leaves in a highly significant way (Karaca, 1998). However, despite the quality and richness of the nutrients that characterize the fruit, regardless the region, a priori the influence of geographic variation on the size is much more intense than the plant organ factor seems insignificant, because regardless the plant organ on which the scale develops (leaf or fruit) Algerian scale remains always larger than Turkish scale. The comparison between the average size of the parasitized and non-parasitized scale for the two countries leads us to deduce that there is a positive relation between the host size and the parasitism because the parasite attributes to itself the most important hosts. Similar results were found in another study in Spain (Pekas et al., 2010). Moreover, the highest parasitism rates of *A. melinus* and *Aphytis lignanensis* (Compere, 1955) are noticed on hosts with the largest cover sizes (Opp & Luck, 1986).

A study carried out by researchers (Yarpuzlu et al., 2008) on lemon trees; showed that *A. melinus* mainly looks for individuals attached to the fruit. These results are in accordance with our results where we found a higher parasitism rate on the fruit than on leaf in the two scale populations (Algerian and Turkish) and confirm the presence of a close connection between the plant organ, the host size and parasitism. The female of *A. melinus* had the capacity to decide the sex of its offspring by attributing male eggs mainly to small size hosts, inferior to 0.39 mm² and female eggs to large size hosts over 0.39 mm² (Pekas et al., 2010; Yu, 1986; Luck & Podoler, 1985). Therefore, it's possible that the scarcity of suitable hosts for the production of females by *A. melinus* in Turkish population compared to that of Algeria, would lead to an evolution towards sex ratios favoring males and consequently a lower parasitism rate. Indeed, the adults of *Aphytis chrysomphali* (Mercet, 1912) from large size hosts were significantly larger and lived almost twice as long as parasitoids from smaller hosts (Desfilis, 2007). On the other hand, coexistence with other predators or other parasites in the orchard can probably compete with *A. melinus* resulting in a decrease in its effectiveness.

Conclusion

The host size is considered to be a key element in the successful biological control of *A. aurantii* because it strongly influences the parasitoid effectiveness. However, it varies considerably depending on the locality where it's situated. Indeed, it depends on the region of the attacked orchard, *A. aurantii* showed large or small measurements. The size variation observed in this work (Algerian scale larger than that of Turkey), is mainly explained by climatic variations between the two countries and its repercussions on phenology and metabolism of the host plant consequently affecting the scale size. In addition, regardless the plant organ on which the scale develops (leaf or fruit), we have found that Algerian scale remains always larger than Turkish scale, then we deduce that the type of plant substrate on which scale grows, doesn't affect the difference in scale size observed between the two localities. A positive relation was noted between the presence of the parasite and the host size, indicating the exploitation of *A. melinus* of high quality hosts (with large size). A parasitism rate of up to 36% is recorded in Algerian scale population while that of Turkey the maximum is about 22%, these results follow those of the size comparison between the two countries where the largest sizes recorded of *A. aurantii* are those coming from Algeria, moreover we think that Algerian scale population offers to the parasite an availability of better quality hosts in terms of size and consequently a higher incidence.

Acknowledgements

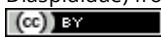
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