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MANAGEMENT OF A MAIN CITRUS PEST BLACK PARLATORIA SCALE PARLATORIA ZIZIPHI (LUCAS) (HEMIPTERA: DIASPIDIDAE) IN THE MEDITERRANEAN BASIN

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ABSTRACT

The scale of black parlatoria, Parlatoria ziziphi (Lucas) (Hemiptera: Diaspididae), causes considerable damage to citrus fruits in the Mediterranean basin. For well managed this pest, we carried out a thorough ecological and behavioral study on the preference of localization and fixation of this pest on the citrus tree throughout the year for two years in six different citrus orchards to know: the lemon, the clementinier and the orange variety Thomson and washington in Tizi Ouzou, sub-humid region of the Mediterranean, in the North of Algeria. This new information on the biology and behavior of *P. ziziphi* could be applied to the definition of a better pest management strategy and to improvement of sampling methodology for monitoring this devastating pest by the good recognition of its place of infestations.

Keywords: Citrus; Parlatoria ziziphi; leaves; fruits; twigs; tree canopy.

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1. INTRODUCTION

The black parlatoria scale, Parlatoria ziziphi (Lucas) (Hemiptera: Diaspididae), is an armored



scale insect considered a major pest of citrus all around the world [1-3]. It is common in the Mediterranean countries [4] and is of economic importance in Egypt [5], France [6], Algeria, Tunisia, Morocco, Italy and Greece [7]. Its populations are particularly significant in the Maghreb countries, mainly in Algeria and Tunisia [8], where it has been considered one of the main citrus pests for many years [9,10,7]. In Algeria, the black parlatoria scale is present in citrus since at least the beginning of the XXth century [11,12].

The damage caused by *P. ziziphi* consists in the weakening and drying of aerial parts of the tree, discoloration of the leaves and fruits, and, most importantly, a reduction of the commercial value of fruit due to the scales fixed on it [13,14]. This cosmetic damage is aggravated by its specific resistance to fruit cleaning [10]. Further, it is one of the most difficult armored scales to control with insecticides [15].

Studies on the bio-ecology of *P. ziziphi* in citrus orchards have been carried out in several countries, such as Spain [16], Sicily [17], Tunisia [18], China [19], Taiwan [2], Greece [20] and Egypt [21]. *Parlatoria ziziphi* attacks all aerial parts of the tree: leaves, twigs, branches and fruits [13-15,22]. Its fixation behavior on the plant is typical of all armored scales, but there are differences between species in spatial distribution on different organs within the host plant. For example, *Aonidiella aurantii* (Maskell) prefers fruits [23], while *Parlatoria pergandii* Comstock prefers the branches inside the tree canopy [24,25].

Work has been published on the black parlatoria scale on the behavior and location of attachment to different tree organs and within host-plant spatial distribution [10,21,26], but such studies generally include only short-term or qualitative observations. Because it is known that the spatial distribution of the armored scales is influenced by factors such as orchard structure or season of the year [27]. The objective of this work is to improve our knowledge of the spatial distribution of *P. ziziphi* on the tree as well as its location on various organs of the plant, determining if the overall preferences change throughout the year and in different orchards. This knowledge is important from a practical point of view for the development of appropriate sampling methods and for improving integrated pest management strategies.

2. EQUIPMENT AND METHODS

2.1. Region studies

The present study was carried out in the wilaya (province) of Tizi Ouzou, located in the north of Algeria (X= $3^{\circ}42^{\circ}56^{\circ}$ ° E - $4^{\circ}39^{\circ}19^{\circ}$ E and Y= $36^{\circ}27^{\circ}6^{\circ}$ N - $36^{\circ}54^{\circ}42^{\circ}$ N), characterized by a temperate climate with a mild winter and is in a sub-humid bioclimatic stage. Mean annual of daily maximum and minimum temperatures are 24.0°C and 13.6°C respectively, with average annual rainfall of 867 mm.

2.2. Sampling

We selected six commercial citrus orchards in full production in different localities in Tizi Ouzou, one lemon orchard (Citrus limon (L.) Burm.) and one orange orchard (Citrus sinensis (L.) Osbeck) and one clementine orchard (Citrus reticulata Blanco) in the locality of Irdjen, one clementine orchard in Chamlal, one lemon orchard and one orange orchard in Chabane locality. Every orchard was sampled at ten-day intervals through a twelve-month period, from January 2014 to December 2015. The sampling methodology consisted of dividing each orchard into nine square-shaped plots with 25 trees each. On each sampling date, we selected randomly two trees per plot. On each tree, we sampled randomly five 20-cm long twigs containing two leaves each, one from each of the four compass points on the external part of the tree canopy and from the interior of the canopy. One mature fruit was also sampled on each orientation when present.

2.3. Statistical Analysis

The differences in population density between organs (leaves and twigs, leaves and fruits, upper and lower side of the leaf, twigs of interior and exterior of the tree canopy, and twigs from the four compass points), were analyzed by two-way ANOVAs for each one of the six orchards and for the total. A two-way ANOVA per season was also carried out the compare the four compass points within each of the four seasons. Data of percentage were [arcsin] transformed to meet normality assumptions. Means were compared using Fisher's least significant difference (LSD) test with the significance level set at α =0.05. All statistical analyses were performed using Statgraphics Centurion XVI [28].

3. RESULTS AND DISCUSSION

3.1. On leaves, twigs and fruits

We compared the abundance of living females of *P. ziziphi* among the three organs of the citrus tree we sampled, leaves, twigs and fruits. The comparison of the overall abundance between leaf and twig showed a strong dominance of *P. ziziphi* individuals on the leaves. The average number of *P. ziziphi* per leaf was 2.44 ± 0.04 , while in a twig this average was 0.233 ± 0.004 (F = 83.8; df = 1, 213; P < 0.0001). This represents that on a leaf there is approximately 10 times more population than on a twig. This strong tendency of individuals of *P. ziziphi* to fix on leaves appears in all six citrus orchards studied: the population abundance on leaves oscillates between 7.8 and 11.7 times higher on leaves than on twigs (Table 1). In any case, the average surface area of a leaf (15-25 cm²) is 3-5 times larger than that of the twig (5-10 cm²).

number of live females per leaf or twig									
orchard –	Leaves		Tw	igs	Factor				
	Mean	SEM^1	Mean	SEM^1	(leaf/twig)	Р			
Lemon-Chabane	2.582	0.064	0.333	0.012	7.8	< 0.0001			
Lemon-Irdjen	3.204	0.137	0.274	0.012	11.7	< 0.0001			
Clementine-Chamlal	0.850	0.023	0.094	0.006	9.0	< 0.0001			
Clementine-Irdjen	3.272	0.129	0.283	0.013	11.6	< 0.0001			
Orange-Irdjen	3.327	0.159	0.291	0.014	11.4	< 0.0001			
Orange-Chabane	1.548	0.043	0.136	0.006	11.4	< 0.0001			
Total	2.44	0.04	0.233	0.004	10.5	< 0.0001			

Table 1. Mean $(\pm \text{SEM}^1)$ population density of *P. ziziphi* on leaves and twigs, expressed as

¹Standard Error of Mean

The population trend of abundance of living females per leaf and twig through the year showed that the differential factor remains during all the months of the year in favor of the leaf. The ratio leaf / twig oscillate through the year with two maxima of up to 15 for the months on April and September (Fig. 1).



Fig.1. Seasonal change through the year in the mean number (± SE) of *P. ziziphi* live female scales on leaves and twigs, the horizontal line represents ratio number 1. Vertical lines represent standard error of the mean

During the three months, October to December, when the mature fruits were present on the trees, we compared the level of infestation per fruit and per leaf. The results show that the population of living females of *P. ziziphi* is always higher on leaves, reaching on average 0.78 \pm 0.11 scales per leaf, while fruits harbor only 0.45 \pm 0.05 scales per fruit (F = 93.79; df = 1, 34; P = <0.0001) (Fig. 2). This ratio leaf/fruit in *P. ziziphi* population changes with the orchard, but always remains in favor of the leaves (Table 2).

Table 2. Mean (\pm SEM¹) population density of *P. ziziphi* on leaves and fruits, expressed asnumber of live females per leaf or fruit

anahand	Lea	ves	Fru	uits	Factor	D	
orchard	Mean SEM ¹		Mean	SEM^1	(leaf/fruit)	Ľ	
Lemon-Chabane	1.256	0.274	0.273	0.103	4.6	< 0.0005	
Lemon-Irdjen	1.607	0.463	0.359	0.104	4.5	< 0.0247	
Clementine-Chamlal	0.810	0.300	0.284	0.118	2.9	< 0.1618	
Clementine-Irdjen	0.954	0.284	0.352	0.110	2.7	< 0.1374	
Orange-Irdjen	0.517	0.119	0.264	0.110	2.0	< 0.2269	
Orange-Chabane	1.107	0.185	0.173	0.093	6.4	< 0.0028	
Total	0.78	0.11	0.45	0.05	1.7	< 0.0001	

¹Standard Error of Mean



Fig.2. Seasonal change through the year in the mean number $(\pm SE)$ of *P. ziziphi* live female scales on leaves and fruits, horizontal line represents ratio number 1, vertical lines represent standard error of the mean

On the upper side of leaves we found an average number of 1.59 ± 0.18 *P. ziziphi* living females, while on the lower side it was only 0.85 ± 0.09 (F = 86.53; df = 1, 213; P < 0.0001). This represents that 65.3% of individuals of *P. ziziphi* are located on the upper face against 34.7% on the underside, or approximately twice as much population of living females on the upper side than on the lower side (Fig. 3). Significantly higher scale population on the upper face of the leaf was also found in five of the six orchards studied (Table 3).

Table 3. Mean (\pm SEM¹) population density of *P. ziziphi* on upper and lower side of the leaf,expressed as number of living females per leaf side

Orchard	Lower side		Uppe	r side	Factor	Р
	Mean	SEM^1	Mean	SEM^1	(upper/lower)	
Lemon-Chabane	1.804	0.286	0.778	0.110	2.3	< 0.0001
Lemon-Irdjen	1.725	0.511	1.479	0.325	1.2	< 0.1131
Clementine-Chamlal	0.532	0.078	0.318	0.070	1.7	< 0.0004
Clementine-Irdjen	2.197	0.526	1.074	0.267	2.0	< 0.0001
Orange-Irdjen	2.397	0.742	0.930	0.231	2.6	< 0.0001
Orange-Chabane	1.019	0.187	0.529	0.076	1.9	< 0.0002
Total	1.59	0.18	0.85	0.09	2	< 0.0001

¹Standard Error of Mean

The monthly evolution through the year in the average number of living individuals per leaf

side showed that the preference for the upper side is not constant but oscillates reaching its maximum in March and the lowest value in September, when the abundance of living females of *P. ziziphi* is similar in both sides of the leaf (Fig. 3).



Fig.3. Seasonal change through the year in the mean number (± SE) of *P. ziziphi* live female scales on upper and lower side of leaves. The horizontal line represents ratio number 1.
Vertical lines represent standard error of the mean

3.2. Inside and outside of the tree

Concerning the abundance of living females of *P. ziziphi* on the interior (center) and exterior (the outer crowns of East, South , North and West orientation) of the tree canopy, the results showed that populations were significantly more abundant at the interior of the tree, averaging 138.0 ± 18.4 individuals per sample unit (one sample unit includes all organs from a particular orientation observed on each sampling date, that is to say, 18 twigs and 36 leaves), whereas in the exterior of the tree we found 80.5 ± 5.0 individuals per sample unit (F = 86.53; df = 1, 210; P < 0.0001). This represents that there is 1.7 times more population in the interior of the tree canopy than at the outside (Fig. 4). This higher density of *P. ziziphi* populations in the interior of citrus trees is not homogeneous in all orchards. In the three orchards of the Chabane and Chamlal regions (which showed the lowest tree density according to shaded surface area of the orchards, see Material and Methods section) the population density of living females of *P. ziziphi* was distributed in a similar way inside and out of the tree canopy, while in the three

orchards of the Irdjen region (with the most dense vegetation) the abundance of *P. ziziphi* was twice as high inside the tree canopy compared with outside (Table 4).

Orchard	Interior		Exte	erior	Factor	D
	Mean	SEM ¹	Mean	SEM ¹	(interior/exterior)	1
Lemon-Chabane	95.2	16.7	99.9	14.6	0.95	< 0.4782
Lemon-Irdjen	200.6	56.1	100.2	24.8	2.00	< 0.0311
Clementine-Chamlal	32.0	5.4	32.3	5.5	0.99	< 0.3593
Clementine-Irdjen	189.0	40.5	101.5	24.9	1.86	< 0.0001
Orange-Irdjen	255.8	76.2	88.6	26.1	2.89	< 0.0004
Orange-Chabane	55.4	12.0	58.9	10.1	0.94	< 0.9048
Total	138	18.4	80.5	5	1.7	< 0.0001

Table 4. Mean (\pm SEM¹) population density of *P. ziziphi* inside and outside the citrus tree canopy

¹Standard Error of Mean

When we represented the monthly evolution through the year in the average number of living females of *P. ziziphi* inside and outside of the tree canopy, the density in the interior of the tree changes periodically reaching two maxima, in April and September, while there is a minimum in June when populations are rather similar inside and outside (Fig. 4).



Fig.4. Seasonal change through the year in the mean number $(\pm SE)$ of *P. ziziphi* live female scales at the inside and outside of the tree canopy. The horizontal line represents ratio number 1. Vertical lines represent standard error of the mean

3.3. Cardinal points differences in abundance

The average number of living female per sample unit on the four compass orientations showed a mean value of 89.1 ± 11.1 scales in the East, 85.3 ± 9.8 in the South, 81.2 ± 8.6 in the North, and 66.3 ± 8.2 in the West. Thus, the East orientation has the highest abundance of living females of *P. ziziphi* with a percentage of 27.7%, South and North are close by with 26.5% and 25.2% respectively, while the West contains the lowest population with 20.6% (F = 14.30; df = 1, 166; P < 0.0001). However, the distribution on the four cardinal directions of the tree canopy varies according to the orchard. In four of the six orchards sampled there were no significant differences among the orientations (Table 5).

Table 5. Mean $(\pm \text{SEM}^1)$ population density of *P. ziziphi* on the four cardinal directions of the

tree									
Orahand	East		North		West		South		Р
Orchard	Mean	SEM^1	Mean	SEM^1	Mean	SEM^1	Mean	SEM^1	
Lemon-Chabane	96.6	15.6	98.9	15.7	63.4	10.2	140.6	24.1	< 0.1216
Lemon-Irdjen	137.4	46.3	121.9	30.1	82.7	24.3	58.7	15.9	< 0.0021
Clementine-Chamlal	38.1	8.0	37.4	9.4	25.3	5.0	28.5	6.6	< 0.2572
Clementine-Irdjen	106.8	31.8	108.2	29.3	80.4	24.1	110.6	27.0	< 0.0450
Orange-Irdjen	95.1	27.1	64.9	17.9	93.1	30.5	101.1	35.8	< 0.1742
Orange-Chabane	59.5	10.3	54.4	11.1	51.1	9.9	70.6	18.0	< 0.0871
Total	89.1	11.1	81.2	8.6	66.3	8.2	85.3	9.8	< 0.0001

¹Standard Error of Mean

The distribution on the four compass points in the population density of living females of *P. ziziphi* changes with the season of the year (Fig. 5). We found that the South direction is the most favorable during the autumn and winter seasons (autumn: F = 4.74; df = 3, 53; P = 0.0034, winter: F = 1.17; df = 3, 53; P = 0.0123), while East, North and South are preferred during the summer (F = 5.76; df = 3, 53; P = 0.0009), with no differences in the spring (F = 0.86; df = 3, 75; P = 0.4620). The West orientation remains the least preferred throughout the four seasons of the year.





(P > 0.05). The horizontal line represents 25% of the population. Vertical lines represent standard error of the mean

In our results, populations of *P. ziziphi* showed a strong dominance on the leaf with a high density on the upper face of the leaf at a rate of 65%. Also, 16,13,29,22 and 30 spoke about the preference of *P. ziziphi* for the leaf, but without giving a precise quantification. Our results are consistent with those of [26] and [30] who point out that most of the *P. ziziphi* population appears on the upper surface of the citrus leaf.

This work also demonstrated that population density of *P. ziziphi* is higher at the interior of the tree canopy compared with the exterior. [16], [10] and [21] also reported a tendency on *P. ziziphi* to fix on the central and shaded branches of trees, with *P. ziziphi* outbreaks being more important in tight and poorly ventilated orchards. Our results revealed that the density of *P. ziziphi* in the center of the tree is not fixed and homogeneous but changes according to the orchard, because several parameters such as orchard layout, tree age and size, pruning system, distance between trees and climatic factors, in particular the high humidity in the center due to poor aeration and the absence of light, may influence the distribution of *P. ziziphi* populations on the tree canopy [10]. Reports that direct exposure to the leaves of the outer crown of the tree can cause significant mortality of first instar larvae of *P. ziziphi*.

The cardinal distribution of *P. ziziphi* populations underlined a great variability depending on the season of the year and the orchard, being the most heterogeneous parameter in our study. Overall, we found that the East quadrant had the highest population and West quadrant the lowest. Our results agree with those of [21] who found 24-28% of *P. ziziphi* females in the East quadrant versus 21-22% in the West.

It was clear in our study that the highest population density on leaves compared with branches or fruit is the most constant or stable parameter of the four aspects studied, because it was maintained during the 12 months of the year and in the six the citrus orchards studied. The distribution inside or outside of the tree canopy and on the cardinal orientations was more variable depending on the month of the year and the orchard considered. The higher abundance of P. ziziphi on some organs or parts of the tree canopy can be explained on one side by to the choice of a place of fixation by neonate larvae that avoid extreme and unfavorable climatic conditions and on the other to differential mortality that occurs along the period of development of the insect. The mobile larvae (crawlers) of armored scales are known for the very limited duration, a few hours, before they settle permanently on the substrate for the rest of their life [31]. The larva relies on a combination of stimuli to locate the fixation site. This stimuli are initially visual and olfactory [32, 33]. The visual stimulus may be influenced by color perception, shape of the host plant and spectral intensity or reflectivity [34]. Positive phototaxis defines an orientation towards a light source [32]. Light appears to be a major factor influencing the direction of wandering in armored scales. Settling by crawlers may be induced largely by thigmotaxis, or response to a contact or mechanical stimulus from crevices, other surface irregularities or contact surfaces, producing in some cases closely packed aggregations of scales [31]. By tasting, the chemical content of the plant is verified by the insect for continuation of feeding [35-37]. All these stimuli can be strongly conditioned by climatic factors such as humidity, temperature and direct sunlight which negatively influence neonate larvae, and which are responsible for decreasing or increasing the population of *P. ziziphi* on an organ or part of the tree [38] reports that the population density of P. ziziphi is negatively influenced by relative humidity and precipitation and positively by temperature. This last climatic factor has a strong influence on the physiological

mechanisms of ectothermic insects [39], and consequently conditions many behavioral strategies [40].

4. CONCLUSION

The information reported by most authors on the distribution of *P. ziziphi* populations on the citrus tree was often based on non-quantified or short-term observations. With this work, we provide new information on the biology and behavior of the black parlatoria scale by determining the changes in its distribution depending on the month of the year and the diversity of citrus orchards. From an applied point of view, the information provided may be applicable to the improvement of the sampling methodology of the black parlatoria scale *P. ziziphi* populations and to define a better strategy of chemical spraying or mechanical pruning for pest management.

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