



**Abundance, seasonality and parasitism of Diaspididae (Hemiptera) on olive trees**

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**Abstract** - Olive culture is a recent activity in Rio Grande do Sul, Brazil with little information on its scale insects. Abundance and seasonality of armored scale insects (Hemiptera, Diaspididae) and their parasitism was evaluated in olive orchard, *Olea europaea* L., cultivar Arbequina, in Caçapava do Sul (30°30'43" S, 53°29'27" W), RS, Brazil. Samples were monthly collected from April 2012 to March 2013. At each occasion 20 trees were drawn and, from each quadrant of their canopies (northern, southern, eastern and western) two branches (20 - 30 cm - length and contained at least 20 leaves) were randomly removed, respectively from the inner part and the outer part of the canopy. We found five species of Diaspididae, *Aonidiella aurantii* (Maskell), *Hemiberlesia cyanophylli* (Signoret), *Acutaspis paulista* (Hempel), *Aspidiotus nerii* Bouché and *Melanaspis obscura* (Comstock). It was not found an unique pattern of abundance, indicating that these species respond differently to temperature variations between seasons. The armored scale insects were evenly distributed among quadrants. Only *H. cyanophylli* showed differences in abundance between the inner and outer branches. We registered parasitism in all Diaspididae species; the total parasitism rate was 9.78%.

**Key-words:** Armored scale insects. Biological control. *Olea europaea*. Seasonal abundance.

**Abundância, sazonalidade e parasitismo de Diaspididae (Hemiptera) em oliveiras**

**Resumo** - A olivicultura é uma atividade recente no Rio Grande do Sul, Brasil, com pouca informação sobre as cochonilhas associadas a esta cultura. Abundância e sazonalidade das cochonilhas-com-escudo (Hemiptera, Diaspididae) e seu parasitismo foram avaliados em olival, *Olea europaea* L., cultivar Arbequina, em Caçapava do Sul (30°30'43" S, 53°29'27" O), RS, Brasil. As amostras foram coletadas mensalmente, a partir de abril de 2012 a março de 2013. Em cada ocasião, 20 árvores foram sorteadas e, de cada quadrante de suas copas (norte, sul, leste e oeste) dois ramos (20 - 30 cm de comprimento e contendo pelo menos 20 folhas) foram retirados aleatoriamente, respectivamente, da parte interna e a parte externa da copa. Foram encontradas cinco espécies de Diaspididae,

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*Aonidiella aurantii* (Maskell), *Hemiberlesia cyanophylli* (Signoret), *Acutaspis paulista* (Hempel), *Aspidiotus nerii* Bouché e *Melanaspis obscura* (Comstock). Não foi encontrado um padrão único de abundância, o que indica que essas espécies respondem diferentemente a variações de temperatura entre as estações. Verificou-se que as cochonilhas se distribuem uniformemente entre os quadrantes. Apenas *H. cyanophylli* mostrou diferenças na abundância entre os ramos internos e externos. Foi registrado o parasitismo em todas as espécies de Diaspididae, a taxa de parasitismo total foi de 9,78%.

**Palavras-chave:** Cochonilhas-com-escudo. Controle biológico. *Olea europaea*. Abundância sazonal.

## Introduction

Globally, the country with the largest olive production is Spain (COI, 2017), and according to Wrege et al. (2015), there is an increasing need to expand production areas due to the increase in commercial demand. In Brazil, according to the Brazilian Olive Farm Institute (IBRAOLIVA, 2017), there are 6,500 hectares of planted area, cultivated by 330 producers, being in Rio Grande do Sul an area of 3,464 hectares of olive groves. As in other regions of the world, scale insects cause major damage by sucking the tree sap and sooty mold formation, which hinders photosynthesis, resulting in some defoliation and reduces the production (COUTINHO et al., 2009). Few control measures are available, pruning the plant canopy, eliminate microclimates, reducing moisture and creating adverse conditions for the development and spraying mineral oil kills scales by suffocation (PRADO et al., 2012).

There are over 84 species of scale insects recorded in olive trees worldwide, 60 belonging Diaspididae (GARCÍA MORALES et al., 2016). In Brazil, Prado et al. (2012) registered ten Diaspididae species in olive crops: *Acutaspis paulista* (Hempel), *Acutaspis scutiformis* Cockerell, *Aspidiotus nerii* Bouché, *Chrysomphalus aonidum* (Linnaeus), *Hemiberlesia rapax* (Comstock), *Parlatoria oleae* (Colvée), *Parlatoria proteus* (Curtis), *Pinnaspis aspidistrae* (Signore), *Pseudaonidia trilobitiformis* (Green) and *Pseudaulacaspis pentagona* (Targioni-Tozzetti). Wolff (2014) included in this relation the species, *Aonidiella aurantii* (Maskell), *Hemiberlesia cyanophylli* (Signoret), *Hemiberlesia lataniae* (Signoret), *Pinnaspis strachani* (Cooley) and *Melanaspis obscura* (Comstock) and Ricalde et al. (2015) included *Comstockaspis perniciosus* (Comstock). Brazilian studies only recorded species of armored scales in olive did not evaluate the seasonal abundance, neither mortality nor parasitism. Scientific studies that address abundance, seasonality and parasitism of insects are fundamental for the improvement of olive crop management, especially due to its recent introduction as an economically important crop in the Brazil. Phillipini et al. (2014) affirm that there is a real possibility that Brazil will increase the olive production chain in order to compete in the international market. With this growth of cultivated areas, information will be needed to support the sustainable management of the crop. This study registered the abundance, seasonality of Diaspididae species and their parasitism rates in olive trees.

## Material and Methods

The study was conducted in Caçapava do Sul (30 ° 30'43 "S, 53 ° 29'27" W), Rio Grande do Sul, in an olive orchard of Arbequina variety, with 0.95 ha and 471 trees, established in 2006. The daily temperature (° C),

rainfall (mm) and relative humidity (%) were obtained from Weather Station of Caçapava do Sul, of Instituto Nacional de Meteorologia. Samples were taken monthly from April 2012 to March 2013 in 20 plants drawn at each occasion. We removed two branches from each quadrant (northern, southern, eastern and western) per tree, respectively from the inner and the outer parts of the canopy, about 1.50 m above the ground, totaling eight branches per plant. Each branch (sample unit) of 20 to 30 cm in length and at least 20 sheets were individually packed into plastic bags, maintained in a refrigerator ( $\pm 4^\circ\text{C}$ ) and examined under stereomicroscope. We recorded the site of the scale insects on the leaf: abaxial leaf side (AB), adaxial (AD), leaf edge (ED) or branch (BR).

The specimens of Diaspididae had the shells removed to record adult with eggs (AEG), adults without eggs (A), first instar (N1) and second instar nymphs (N2). Males of Diaspididae were recorded through their empty shell because of its emergence or when the adult had not yet emerged. All species of scale insects were recorded - live individuals (L), dead, without evidence of parasitism (K), infested with larvae of parasitoids (LA), with parasitoid pupae (PU), perforated after the emergence of parasitoids (D) and meconium, feces left by the parasitoid after emergence (ME).

Scales were identified by Dra.Vera Regina dos Santos Wolff and deposited in collection of Museu de Entomologia Ramiro Gomes Costa (MRGC), of Departamento de Diagnóstico e Pesquisa Agropecuária- DDPA, da Secretaria Estadual de Agricultura, Pecuária e Desenvolvimento Rural - SEAPDR, Porto Alegre, Brazil.

The seasonal abundance of each scale species was analyzed by Kruskal-Wallis test and compared by Dunn test. The abundance of species occurring in only two seasons were compared by Mann-Whitney test, as well as the average number of individuals in the inner and outer branches per tree. The mean number of individuals per quadrant, tree and site in the sample unit (AB, AD, BR or ED) was analyzed by Kruskal-Wallis test. The parasitism rate included the scale insects in the conditions D, PU, LA and ME, the difference in the proportion of parasitism among species of scale insects was tested with the heterogeneity  $\chi^2$ . The average of live scale insects (LIVE), dead parasitized (DP) dead not parasitized (DNP) was analyzed by Kruskal-Wallis and compared by Dunn. It was not possible to identify the parasitoids species with this methodology because the scales had their shells removed, preventing its further development. The association of meteorological factors to the monthly average number of individuals per tree was evaluated by Pearson correlation test. The minimum level of significance adopted for all analyzes was 5% and the software used was Bioestat 5.0<sup>®</sup> (AYRES et al., 2007).

## Results and Discussion

Five Diaspididae species were present in olive trees, *A. aurantii*, *H. cyanophylli*, *A. paulista*, *A. nerii* e *M. obscura*. Their mean number of individuals ( $\pm$  SE) per sampling occasion during the study were: *A. paulista* ( $33.5 \pm 2.41$ ), *A. aurantii* ( $15.4 \pm 5.45$ ), *H. cyanophylli* ( $9.1 \pm 1.02$ ), *A. nerii* ( $1.1 \pm 0.53$ ) and *M. obscura* ( $0.02 \pm 0.07$ ).

*Acutaspis paulista* is Neotropical, occurring on several plant species in Argentina and Brazil (CLAPS; WOLFF, 2003; GARCÍA MORALES et al., 2016). In Brazil, this species occurs especially in citrus and was recently recorded on *Olea europaea* L. in Santa Catarina and Rio Grande do Sul, (WOLFF, 2014). In Argentina, it was observed on olive trees in the province of Catamarca (FUNES, 2012).

*Hemiberlesia cyanophylli* was observed in 70 families of host plants in the world (GARCÍA MORALES et al., 2016). In Brazil was found on olive trees and in over 40 other plants (CLAPS; WOLFF, 2003; WOLFF, 2014).

*Aspidiotus nerii* had already been observed in *O. europaea* in Brazil and Argentina (CLAPS; WOLF, 2003). It is considered a cosmopolitan species with a wide range of host plants; it first register in olive was in Italy in 1868 (GARCÍA MORALES et al., 2016).

*Melanaspis obscura* was also recorded in olive trees in Brazil by Wolff (2014).

We found different patterns of abundance in relation to the seasons (Table 1), indicating that these species respond differently to temperature variations.

**Table 1.** Seasonal mean abundance by species ( $\pm$  SE) of Diaspididae sampled by season on olive trees. Caçapava do Sul, RS, from April 2012 to March 2013.

Taxon	Autumn	Winter	Spring	Summer
<i>Acutaspis paulista</i>	47.5 $\pm$ 9.24 bc*	41.8 $\pm$ 8.37 b	111.8 $\pm$ 21.91 ab	201.1 $\pm$ 54.37 a
<i>Aonidiella aurantii</i>	75 $\pm$ 13.14 a	29 $\pm$ 8.91 b	43.6 $\pm$ 12.84 ab	37.3 $\pm$ 21.63 b
<i>Hemiberlesia cyanophylli</i>	13.6 $\pm$ 4.20 b	19 $\pm$ 3.67 ab	33.8 $\pm$ 7.72 a	43 $\pm$ 7.71 a
<i>Aspidiotus nerii</i>	0.4 $\pm$ 0.22 b	1.8 $\pm$ 0.62 b	0.5 $\pm$ 0.23 b	10.2 $\pm$ 1.77 a
<i>Melanaspis obscura</i>	0	0	0.7 $\pm$ 0.29 a**	1.3 $\pm$ 0.33 a

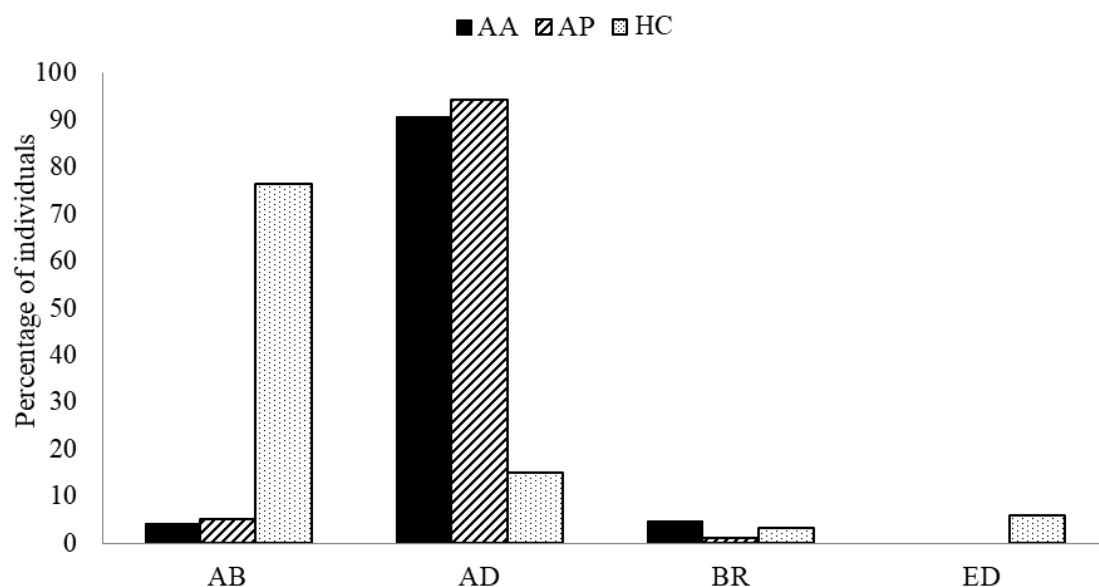
\* Means followed by the same letter on the lines (the same species between stations) do not differ by Kruskal-Wallis test ( $P < 0.0001$ ); \*\* no significant difference using the Mann-Whitney test ( $P < 0.0001$ ).

In *A. paulista*, only the summer and winter differ in terms of number of individuals ( $H = 15.0988$ ,  $df = 3$ ,  $P < 0.05$ ), whereas *A. aurantii* was significantly more abundant in the autumn in relation to winter and summer ( $H = 14.7168$ ,  $df = 3$ ,  $P < 0.05$ ).

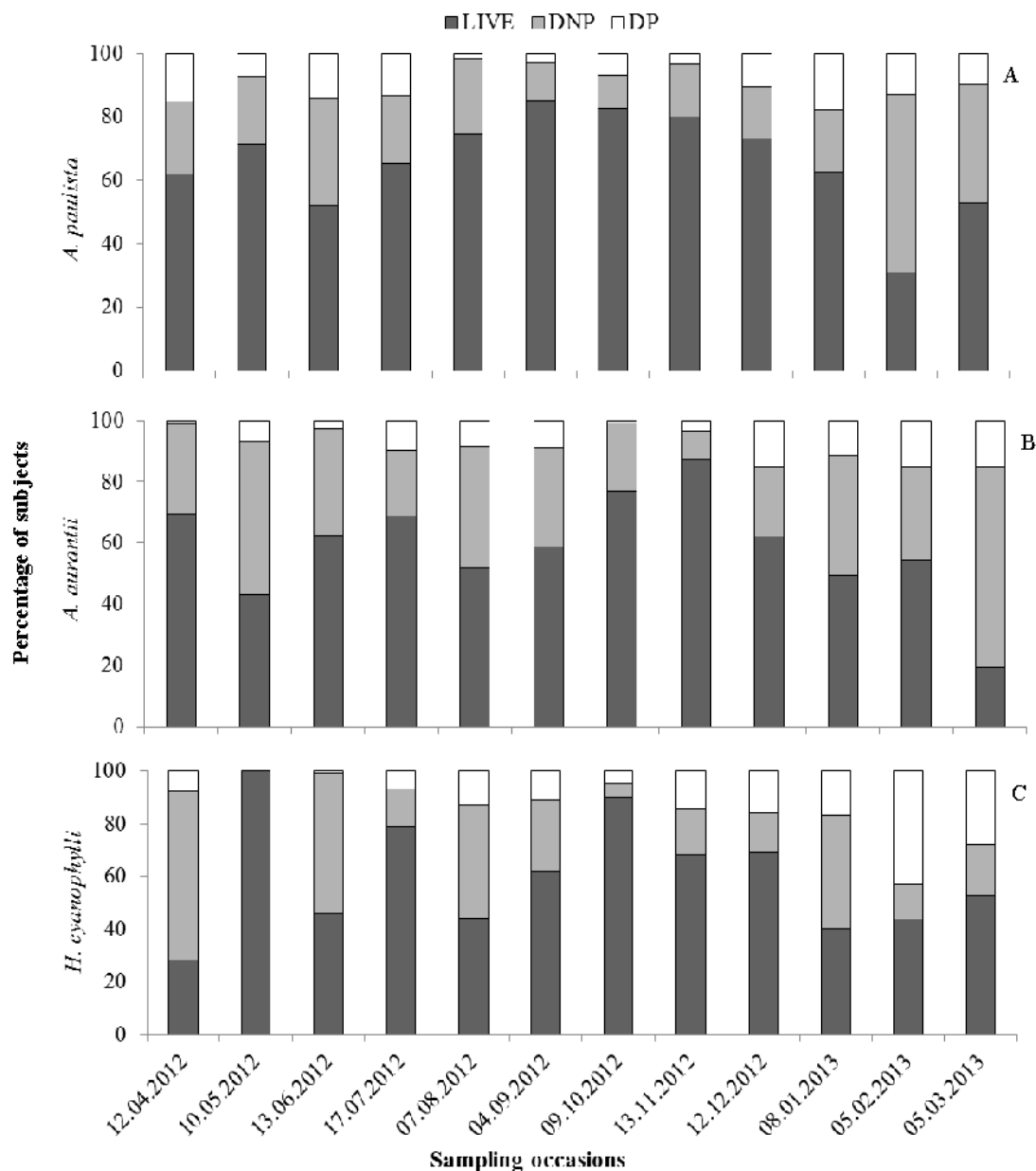
Differently, *H. cyanophylli* had lower abundance in the autumn, distinct from verified in the spring and summer ( $H = 17.7158$ ,  $df = 3$ ,  $P < 0.05$ ). Individuals of *A. nerii* were more abundant in summer ( $H = 32.9129$ ,  $df = 3$ ,  $P < 0.05$ ). *Melanaspis obscura* was observed only in spring and summer, with similar abundance in both seasons ( $Z (U) = 1.3525$ ,  $P = 0.0881$ ). The summer, followed by spring, seem to be the most favorable seasons to four species of scale insects on olive trees, and the temperature had the strongest influence as the lowest ( $r = 0.7325$ ,  $P = 0.0067$ ), average ( $r = 0.7772$ ,  $P = 0.0029$ ) and highest temperature ( $r = 0.7388$ ,  $P = 0.006$ ) correlated significantly and positively with the mean number of individuals. The relative humidity in Caçapava do Sul, always above 70%, is not a limiting condition in the environment for scale insects.

The average number of first instar nymphs (N1) of *A. paulista*, *A. aurantii* and *H. cyanophylli* per tree in each sampling occasion did not differ throughout the year (*A. aurantii* -  $H = 10.5352$ ,  $df = 11$ ,  $P = 0.483$ ; *A. paulista* -  $H = 8.668$ ,  $df = 11$ ,  $P = 0.6525$ ; *H. cyanophylli* -  $H = 2.9447$ ,  $df = 11$ ,  $P = 2.9447$ ). In the N2 phase, *A. paulista* was more abundant from October to March ( $28.6 \pm 13.03$ ) compared with April to September ( $4.6 \pm 2.20$ )

( $H = 49.7564$ ,  $df = 11$ ,  $P < 0.0001$ ). *Hemiberlesia cyanophylli* was more abundant in September and October, but did not exceed the average of five individuals ( $H = 25.9354$ ,  $df = 11$ ,  $P < 0.01$ ). The mean number of *A. aurantii* was similar throughout the year ( $H = 17.5516$ ,  $df = 11$ ,  $P = 0.0926$ ). The monthly mean number of second instar nymphs of these two latter species was fewer than 20 individuals per sampling occasion. The average number of *A. paulista* adults was significantly higher in June, and from October to March ( $20.2 \pm 7.19$ ), compared to other months ( $5.4 \pm 1.51$ ) ( $H = 39.1843$ ,  $df = 11$ ,  $P < 0.001$ ). In *A. aurantii*, February was the only month who differed from the others, with the lowest average abundance ( $H = 24.805$ ,  $df = 11$ ,  $P < 0.0001$ ). Adults of *H. cyanophylli*, on the other hand, were more abundant from December to March ( $11.3 \pm 1.37$ ), compared to other months ( $3.4 \pm 1.49$ ) ( $H = 60.2361$ ,  $df = 11$ ,  $P < 0.0001$ ). Throughout the sampling period, eggs of females were recorded in three species, but no difference was detected in abundance among the months (*A. aurantii* -  $H = 2.5572$ ,  $df = 11$ ,  $P = 0.9954$ ; *A. paulista* -  $H = 11.1249$ ,  $df = 11$ ,  $P = 0.4329$ ; *H. cyanophylli* -  $H = 12.3569$ ,  $df = 11$ ,  $P = 0.3374$ ). There was no evidence of a development standard for diaspidid species, or a way to determine the number of generations throughout the year, since the peak of abundance of each stage was not clear enough for this. The short sampling period and the small number of individuals of each species observed in the different stages may have contributed for this. In Uruguay, Asplanato; García (2001), in the same crop, obtained similar results for *A. aurantii*, an increase in the spring, a second in the summer and the longest in the fall. During this study, the proportion of individuals of *A. aurantii*, N1 and N2 in stages remained constant at around 44%. Asplanato; García (2001) found higher proportions of N1 + N2 around 90% and 70%, respectively. After two years of sampling, both works showed that the peak of these nymphal stages indicated the beginning of a new generation.



**Figure 1.** Percentage (%) of Diaspididae individuals, *Aonidiella aurantii* (AA), *Acutaspis paulista* (AP) and *Hemiberlesia cyanophylli* (HC), seen in the abaxial surfaces (AB), adaxial (AD), the branches (BR), and the edge of the sheets (ED) in olive trees. Caçapava do Sul, RS, from April 2012 to March 2013.



**Figure 2.** Percentage of individuals *Aonidiella aurantii* (A), *Acutaspis paulista* (B) and *Hemiberlesia cyanophylli* (C), live (LIVE), dead not parasitized (DNP) and dead parasitized (DP), for sampling occasion in olive trees. Caçapava do Sul, RS, from April 2012 to March 2013.

Scale insects occur evenly among the quadrants and between the inner and outer part of the olive tree canopy. The amount of scale insects present in the inner and outer branches of the crown did not differ for *A. paulista* ( $Z(U) = 1.0076$ ,  $P = 0.1568$ ), *A. aurantii* ( $Z(U) = 1.1698$ ,  $P = 0.121$ ), *A. nerii* ( $Z(U) = 0.3722$ ,  $P = 0.3549$ ) and *M. obscura* ( $Z(U) = 0.2343$ ,  $P = 0.4074$ ). Since *H. cyanophylli* showed a higher average in the inner ( $5.8 \pm 0.73$ ) than in the outer branches ( $3.3 \pm 0.63$ ) ( $Z(U) = 2.8862$ ,  $P < 0.05$ ). There was no significant difference in the distribution of the average number of individuals in the four quadrants ( $H = 4.3468$ ,  $df = 3$ ,  $P = 0.2264$ ). Regarding *A. aurantii*, Rodrigo; García (1994), in Valencia, Spain, also found similar numbers in the inner and

outer branches, as well as among quadrants in citrus. We did not find in any species differences regarding site and their developmental stages ( $P > 0.05$ ). Comparing the total population on the plant parts, it was found that *A. aurantii* and *A. paulista* were more abundant in the adaxial side of leaves (*A. aurantii* -  $H = 107.4115$ ,  $df = 3$ ,  $P < 0.0001$ ; *A. paulista* -  $H = 260.6877$ ,  $df = 3$ ,  $P < 0.0001$ ). *Hemiberlesia cyanophylli* was more frequent on the abaxial side ( $H = 110.8794$ ,  $df = 3$ ,  $P < 0.0001$ ) and was the only species observed on the edges of the leaves. The three species (*A. aurantii*, *A. paulista* and *H. cyanophylli*) were found on the branches; however, the number of individuals at this site was very small compared to those found on the leaves (Figure 1). The species *A. nerii* and *M. obscura* showed similar distribution on the different parts of the plant (*A. nerii* -  $H = 3.3117$ ,  $df = 3$ ,  $P = 0.1909$ ; *M. obscura* -  $H = 0.1575$ ,  $df = 3$ ,  $P = 0.9243$ ). The highest number of individuals of *A. aurantii* on the adaxial side may be due to the positive phototropism pointed out by Rodrigo; García (1994).

The total parasitism rate was 9.78%. This rate did not differ significantly among species of scale ( $\chi^2 = 0.018665$ ,  $df = 4$ ,  $P < 0.05$ ). *Aspidiotus nerii* and *M. obscura* had the highest rates, 24.3% and 10.3%, respectively. However, this result should be seen with caution due to the small number of individuals of both species sampled in the period, 255 of *A. nerii* and 39 of *M. obscura*. In relation to others species, we sampled 8,041, 3,071 and 2,186 individuals respectively to *A. paulista*, *A. aurantii* and *H. cyanophylli* and the parasitism rates were 9.8%, 7.4% and 11.9%.

The monthly analysis of mortality was made only with the populations of *A. aurantii*, *A. paulista* and *H. cyanophylli*, due to the small number of individuals sampled from the other species. *Acutaspis paulista*, in January, the average DP is equivalent to DNP, as in the rest of the months did not differ significantly ( $H = 29.252$ ,  $df = 11$ ,  $P < 0.0001$ ) (Figure 2A).

During the sampling period, the number of individuals of *A. aurantii*, DNP and DP was similar ( $H = 10.951$ ,  $df = 11$ ,  $P = 0.4474$ ) (Figure 2B). This did not occur in relation to *H. cyanophylli*, the number of DP and DNP individuals differ between months ( $H = 38.1198$ ,  $df = 11$ ,  $P < 0.0001$ ). Dead individuals of this species were not found in May and June. One DP was recorded in the period from October to December and in March the proportion of DNP and DP was similar, while in February the number of DP was three times that DNP (Figure 2C).

## Conclusion

Five species of Diaspididae are reported on olive tree, *Aonidiella aurantii*, *Hemiberlesia cyanophylli*, *Acutaspis paulista*, *Aspidiotus nerii* and *Melanaspis obscura*. They are more abundant in spring and summer. The temperature affects the abundance of scale insects. The scales are equally distributed among the quadrants of the olive tree canopy. All species of armored scales were parasitized.

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