Leaf consumption and oviposition of *Palpita forficifera* (Lepidoptera: Crambidae) on different olive cultivars

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ABSTRACT

The incidence of the olive caterpillar *Palpita forficifera* is among the main phytosanitary problems in olive groves in Brazil, and the use of resistant cultivars can be a useful tool to be incorporated into an integrated management strategy of this pest. The objective of this study was to evaluate the consumption and oviposition of *P. forficifera* on three olive cultivars in laboratory bioassays, in order to identify a potential antixenosis-type resistance. The cultivars evaluated were Arbequina, Arbosana and Koroneiki. Oviposition free choice tests were carried on young trees of the cultivars, with 7 days old adults. For consumption, detached leaves from the different cultivars were used in no choice and free choice tests with 5th instar larvae. The oviposition of *P. forficifera* was similar in all three cultivars. As for consumption, in no choice test, Arbequina had a higher dry mass consumed, yet, in free choice tests, no significant difference was observed in the fresh and dry mass consumed on the three cultivars. These results suggest that none of the cultivars expressed antixenosis-type resistance.

Keywords: *Olea europaea*; non-preference; olive caterpillar; integrated pest management; host plant resistance.

INTRODUCTION

Palpita forficifera Munroe (Lepidoptera: Crambidae) is a moth associated to Oleaceae plants in Latin America. The adult is 23 to 30 mm wide, white-colored, with small dark spots and a brown stripe along the anterior margin of the first pair of wings. Its larvae feed mainly on young sprouts of olive trees (*Olea europaea* L., Oleaceae). It has been considered a key pest affecting olive groves in the Brazilian states of Rio Grande do Sul and Santa Catarina (Ricalde *et al.*, 2015; Carmona, 2018; Castilhos & Brugnara, 2019), and Uruguay (Leoni *et al.*, 2013).

In Brazil, official olive acreage from 2019 harvest season states that 1,424 hectares were harvested, 63% in Rio Grande do Sul (IBGE, 2021). Unofficial surveys point that Brazilian cultivated area were 6,500 hectares in 2017, and estimated to be 10,000 in 2020 (Ibraoliva, 2021). In Santa Catarina, the cultivated area is near 75 hectares[2]. Uruguay cultivates near 9,000 hectares with olive, with a projected potential of 10,000 tons of olive oil production (Asolur, 2021).

Infestation of olive shoots by *P. forficifera* increases from November to February in the South of Brazil (Ricalde *et al.*, 2014), as air average temperature rises. Young leaves and branches consumption by larvae reduces leaf area and limits the growth of branches responsible by flowering in the next season. The consumption of fruit is occasional (Leoni *et al.*, 2013).

Despite the unknown economic threshold for this pest,

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growers use to prevent high infestations mainly by insecticide sprays and cultural practices. In order to implement an integrated pest management system, additional control measures are necessary, which may include the use of cultivars with genetic resistance. Plant resistance to insects is usually categorized in three types: antibiosis, antixenosis and tolerance (Baldin *et al*, 2019a). As stated by Smith (2005), antibiosis is characterized by an adverse effect in insects' biological parameters, antixenosis occurs when insects' behavior is adversely affected and tolerance is expressed when the plant is less damaged than susceptible ones in the same level of infestation, without interference on insects' biology or behavior.

Due its outcrossing nature, olive populations have a high level of genetic variation, and can respond differently in the field against the incidence of *P. forficifera*. So, it's important for growers to know not only the yield and quality of olive and oil, but also what pests and diseases will affect the trees when establishing an olive grove (Guerin *et al.*, 2000). Given the scarcity of information about resistance of olive cultivars to *P. forficifera*, studies that subside the use of genetic resistance in olive pest's management are expected. Therefore, in order to contribute with the knowledge on *P. forficifera* bioecology and management in olive groves, this work aimed to evaluate the leaf consumption and oviposition preference of *P. forficifera* on three olive cultivars in laboratory bioassays.

MATERIAL AND METHODS

Insects

The insects used in the bioassays were from a laboratorial colony stablished from larvae collected on an olive orchard composed by different cultivars at Epagri/Cepaf – Centro de Pesquisa para Agricultura Familiar, Chapecó, SC, Brazil (27°05'13"S, 52°38'12" W), and kept at 25 ± 2 °C temperature, 60 ± 10% relative humidity and 14 hours photophase. Larvae were fed *ad libitum* with leaves from the alternative host *Ligustrum lucidum* Ait. (Oleaceae), primarily to avoid pre-imaginal conditioning of the insects used in the bioassays and also because of its easy availability and great suitability to *P. forficifera*. Larvae were reared in squared (11 cm x 11 cm) transparent polystyrene containers up to 2nd instar, and afterwards were individualized in glass tubes until pupation. The pupae were removed and placed on transparent plastic cages (15 cm diameter x 18 cm high) for adults' emergence, in a density of 20 couples/cage. To compose the couples, pupae were separated by sex as described by Butt & Cantu (1962). The top of the cage was closed with a tule fabric, and above it, a moist filter paper disc (10 cm diameter) was placed to serve as oviposition substrate. After oviposition, the filter paper containing eggs was then placed in the polystyrene containers for hatching of larvae and start of a new cycle.

Oviposition bioassay

Potted young trees of the cultivars Arbequina, Arbosana and Koroneiki with approximately 40 cm high, 1.5 years old and approximate leaf area were equidistantly disposed in a perlite filled 12 L plastic pot and covered with a tulle fabric cage (27 cm diameter x 50 cm high) constituting a choice arena with the three cultivars. In each arena, 20 couples of *P. forficifera* adults with approximately 7 days old were released and were kept in touch with the three cultivar trees for 72 h. Water and a 15% honey solution were offered to adults in separate cotton dishes placed in the center of the arena. After this period, the trees were removed from the cage and the number of eggs laid in each cultivar was counted using a binocular stereomicroscope. Because the leaf area of each three was not precisely the same, the leaves of each tree were detached and the leaf area was measured using a portable leaf area meter model CI-202 (CID Bio-Science), in order to estimate the oviposition density (number of eggs per 100 cm^2). The bioassay was carried in climate-controlled room (25 ± 2 °C temperature, 60 ± 10% relative humidity and 14 hours photophase). Two oviposition trials were carried. In each trial, it was used five replicates, being each replicate composed by one choice arena containing the different olive cultivars infested with 20 couples.

Consumption bioassays

To evaluate the larvae leaf consumption on different olive cultivars, bioassays with no-choice and free-choice were carried in laboratory (25 ± 2 °C temperature, 60 ± 10% relative humidity and 14 hours photophase) with the cultivars Arbequina, Arbosana and Koroneiki, whose leaves were obtained from a 7 years old olive orchard located at Epagri/Cepaf experimental area.

In no-choice test, thirty 5th instar larvae were placed individually in glass tubes (2.5 cm diameter x 8.5 cm heigh) and fed with an average amount of 650 mg of fresh leaves. We opted to use 5th instar larvae because most of leaf

consumption occurs on the later instars, and in the early instars (1rst and 2nd) larvae scraps the abaxial epidermis and as result the upper epidermis overly dries up, what makes evaluations difficult. Because the offered fresh mass in the cultivars and replicates was approximate but not precisely the same, leaves from each replicate were previously weighted in a precision scale and recorded before offering. The tubes were closed with hydrophobic cotton, and after 48 h, the larvae were removed from tubes and remaining leaves were weighted with a precision scale to evaluate the consumption. The experimental design was completely randomized, with 30 larvae per treatment (cultivar).

For the free-choice test, the offered amount of fresh leaves from each cultivar averaged 245 mg. Leaves from each cultivar were weighted and disposed equidistantly in glass dishes (14.5 cm diameter x 2 cm high), constituting a choice arena. A 5th instar larva was placed in the center of each arena, and the remaining leaves were weighted after 48 h to evaluate the consumption on each cultivar. It was used 30 replicates, being each replicate composed by one choice arena with leaves from the different olive cultivars.

For both consumption tests, it was evaluated the absolute (mg) and relative (%) fresh mass consumed and the dry mass (mg) consumed. On the free-choice test, the global consumption rate (%) on each cultivar was calculated. In order to determine the natural humidity loss of the leaves during the 48 h of each test, an aliquot composed by 5 replicates without larvae were used. The leaves were weighted at the beginning of the tests and after 48 h. The humidity loss (UL%) was calculated by the formula: $UL\% = 100 - ((M/FM)\ x\ 100)$, where: FM=fresh mass; M=mass after 48 h. The weight of the remaining leaves in each cultivar was corrected taken into account their respective UL and subtracted from the offered fresh mass to obtain the consumed fresh mass. After that, the aliquot leaves were taken to an incubator at 65 °C for 48 h and weighted to obtain the dry mass rate (DMR), dividing dry mass by fresh mass. The consumed fresh mass was multiplied by the DMR to obtain the consumed dry mass on each cultivar (Paron & Lara, 2001).

Statistical analyses

Dataset was submitted to joint analysis of variance. The normal distribution of residues was verified by Shapiro-Wilk test, and the homoscedasticity of variance between treatments was verified by Bartlett's test. When these assumptions were met, and if the cultivars effect was significant, the averages were compared by Tukey's test ($p < 0.05$). In cases that ANOVA assumptions were not met, data were subjected to Kruskal-Wallis non-parametric test. The statistical analyses were performed with the software R version 4.0.3 (R Development Core Team, 2020), using 'agricolae' package (Mendiburu 2020).

RESULTS

The oviposition of *P. forficifera* was similar in the three cultivars evaluated. For Arbequina, Arbosana and Koroneiki, the average number of eggs verified in each tree was 17.9, 19.4 and 17.2 respectively (Figure 1A). As for oviposition density, no significant difference was observed among cultivars, with 18.8, 17.2 and 13.7 eggs 100 cm^{-2} of leaves for Arbequina, Arbosana and Koroneiki, respectively, showing that *P. forficifera* females have no preference and can oviposit equally on the three cultivars (Figure 1A).

In no choice consumption tests the absolute fresh mass consumed by *P. forficifera* fifth instar larvae was 203.24 mg in Koroneiki, 217.33 mg in Arbosana and 235.02 mg in Arbequina, and no significant difference was observed between cultivars (Figure 1B). The relative fresh mass consumption rate was similar for all cultivars, with 37.64% in Arbequina, 31.35% in Arbosana and 32.08% in Koroneiki (Figure 1C). The dry mass consumed was significantly higher in Arbequina (112.81 mg) in comparison with Arbosana (84.76 mg) and Koroneiki (85.36 mg) (Figure 1D). The DMR verified in the aliquots were 0.48, 0.39 and 0.42 for Arbequina, Arbosana and Koroneiki, respectively in no choice test.

For the free choice test, no significant difference in the consumption was observed among cultivars in all parameters evaluated. The fresh mass consumed was 66.98 mg in Arbequina, 86.93 mg in Arbosana and 87.91 mg in Koroneiki (Figure 1B), corresponding to a consumption of 27.28, 33.16 and 37.78%, respectively, of the fresh mass offered (Figure 1C). The dry mas consumed was equivalent in the three cultivars with 26.12 mg in Arbequina, 36.51 mg in Arbosana and 35.16 mg in Koroneiki (Figure 1D). For the free choice test, the DMR was 0.39 for Arbequina, 0.42 for Arbosana and 0.40 for Koroneiki. The global consumption of each larva was composed by 37.25% of Arbosana, 34.72% of Koroneiki and 28.03% of Arbequina, with no significant difference among cultivars (Figure 1E).

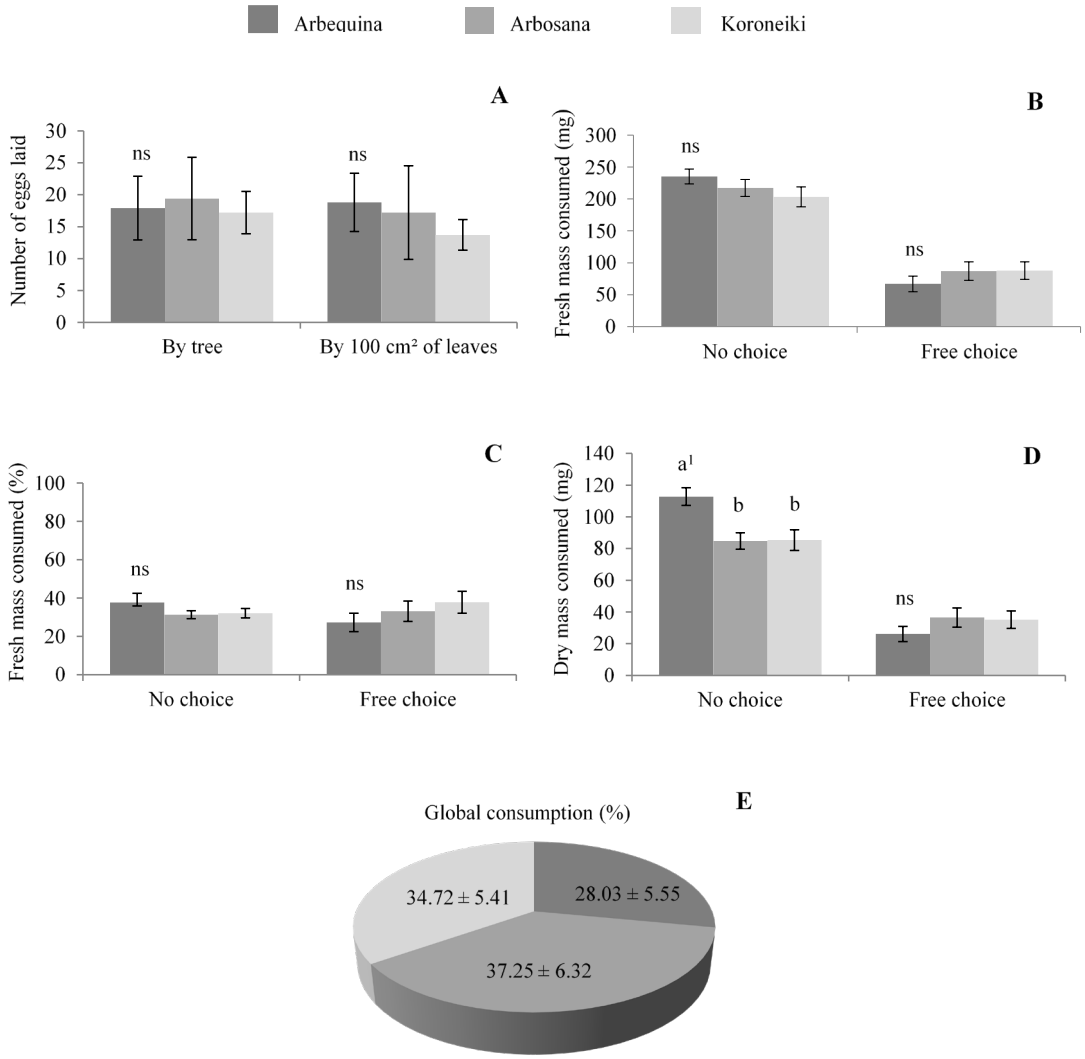

Figure 1: Oviposition and consumption of *Palpita forficifera* on different olive cultivars. Number of eggs and oviposition density on free choice test (A); Absolut fresh mass (mg) consumed in no choice and free choice tests (B); Relative fresh mass (% of mass offered) consumed in no choice and free choice tests (C); Absolute dry mass (mg) consumed in no choice and free choice tests (D); Percentage of the global consumption (mean ± SE) of young olive leaves on different cultivars in free choice test (E).

ns= Difference between columns are not significant (α=0.05).
[1]Columns with different letters differ statistically by Tukey test, α=0.05.
(A) By tree: df (treatment, residue) =2.26; p-value=0.95. By 100 cm^{-2} of leaves: df (treatment, residue)=2.26; p-value=0.763. (B) No choice test: df (treatment, residue)=2.87; p-value=0.26. Free choice test: df=2; p-value=0.55. (C) No choice test: df (treatment, residue)=2.87; p-value=0.079. Free choice test: df= 2; p-value= 0.43. (D). No choice test: df=2.87; p-value=0.0009. Free choice test: df =2; p-value=0.439. (E) df = 2; p-value=0.448.

DISCUSSION

Antixenosis resistance is usually defined as a non-preference reaction of insects to resistant plants, which are less used for food, oviposition substrate and shelter, resulting in the selection of an alternate hostplant (Smith, 2005). Despite the antixenosis concept is well accepted among researchers in the field of plant resistance to arthropods, in some cases, extreme antixenosis may lead insects to inanition and dead, what makes difficult to differentiate antixenosis from antibiosis (Stout, 2013; Baldin *et al.*, 2019b). The causes of antixenosis can be morphological, i.e., thickened epidermal layers, waxy deposits on leaves, stems, high density of trichomes; or chemical, such as insufficient levels of nutrients to stimulate feeding and presence of allelochemicals that repel or deter feeding and oviposition (Smith, 2005; Baldin *et al.*, 2019b).

Olive is considered one of the main hosts for *P. forficifera* because its leaves provide nutrition and energy for larval development (Ricalde *et al.*, 2015; Scheunemann *et al.*, 2019). The chemical constitution of olive leaves from a groove located in the South of Brazil was evaluated by Cavalheiro *et al.* (2014), which found that leaves had 19.5% of total carbohydrates, 8% of lipids and 12% of proteins, in addition to several chemical elements as potassium, calcium, sulfur, phosphorus and zinc. Despite chemical analyses from leaves were not carried in our study, no substantial differences in the digestibility and chemical traits of leaves are expected, since larvae consumption was similar in the three cultivars (Kleine *et al.*, 2011).

The three cultivars evaluated in this work are among the most cultivated ones by olive growers in Brazil, and their production is destinated mostly to oil production (Croce *et al.*, 2016). The cultivars Arbequina and Arbosana are originated from Spain, while Koroneiki has Greece as its center of origin (Oliveira *et al.*, 2012). Besides the difference on the center of origin, some differences in architectural and morphological characteristics are pointed in the literature between these cultivars. As stated by Coutinho *et al.* (2015), the canopy density of Arbosana is higher in comparison to Arbequina and Koroneiki, and some differences in leaf size and longitudinal blade curvature are also pointed. As stated by the authors, Arbequina and Arbosana have medium size leaves with epinastic longitudinal curvature, while Koroneiki have smaller leaves with hyponastic curvature. Even though those differences, the oviposition and consumption of *P. forficifera* was similar in the three cultivars on free-choice tests, evidencing that those specific morphological characteristics on leaves don't lead to the expression of antixenosis-type resistance.

In no choice test, the significant higher dry mass consumed by *P. forficifera* larvae in Arbequina may be explained by its respective higher DMR. Notwithstanding, since in the free choice test no significant difference in consumption was verified, the antixenosis can't be supported for any cultivar. It is well known that chemical composition and the production of defense secondary metabolites in plants can influence the herbivory rate of insects (Descombes *et al.*, 2020), however these attributes were not evaluated in our study, but should be considered in the future in order to explain and justify possible effects of cultivars on *P. forficifera.*

Usually, insects prefer to oviposit in substrates that ensure the high performance for their offspring and the species survival (Thompson, 1988). Based on that, our results suggest that *P. forficifera* can stablish and increase its population at the same rate in all three cultivars, since the oviposition and consumption was equal on all cultivars. High levels of infestation by *P. forficifera* larvae were verified in olive orchards from cultivars Arbequina, Arbosana and Koroneiki in the state of Rio Grande do Sul (Ricalde *et al.*, 2014), corroborating with the findings from our study that this pest can oviposit and consume on those three cultivars without any restriction.

Information about the resistance of olive cultivars to *P. forficifera* are scarce. Scheunemann *et al.* (2019) investigated the biology of *P. forficifera* on the three cultivars evaluated in our study (Arbequina, Arbosana and Koroneiki) under laboratory conditions and found that cultivar Arbosana was less suitable for this pest's development in consequence of the lower larval viability and females' fecundity, indicating a possible antibiosis effect. In addition, Nazari *et al.* (2014) investigated some biological characteristics of *Palpita unionalis* (Hübner) (Lepidoptera: Pyralidae), a species near to *P. forficifera*, on different olive cultivars, including Koroneiki which was also evaluated in our study, and found no significant differences among the life table parameters. In Egypt, some olive cultivars showed antixenosis to *P. unionalis*, since differences in preference for oviposition were observed in orchards composed by varied cultivars (Hegazi *et al.*, 2012). Despite *P. unionalis* and *P. forficifera* are close species, their comparison must be cautious since they occur in different regions of the world with different environmental conditions that can interfere in arthropod/plant interaction.

Because commercial cultivation of olive is relatively recent in Brazil, few studies about *P. forficifera* were carried out so far, and the little information available is mostly focused on bioecology and chemical control (Ricalde *et al.*, 2014; Castilhos & Brugnara, 2019; Scheunemann *et al.*, 2019). Despite antixenosis resistance was not verified for Arbequina, Arbosana and Koroneiki, in our study, the use of resistant cultivars is an effective alternative that can be helpful in *P. forficifera* management. Given this, future research that include evaluation of other larval instars and other possible types of resistance are encouraged, especially in field conditions, in order to identify possible resistant materials to be incorporated in olive breeding programs and in the integrated and sustainable control of this pest in olive orchards.

CONCLUSION

The olive cultivars Arbequina, Arbosana and Koroneiki tend not to express antixenosis-type resistance against *P. forficifera*, since no preference for oviposition neither difference in leaf consumption was observed.

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