*Oecologia Australis*
25(1):80-89, 2021
https://doi.org/10.4257/oeco.2021.2501.08

# FEEDING PREFERENCE AND BIOLOGICAL TRAITS OF *Panonychus ulmi* ON LEAVES OF APPLE AND GRAPEVINE

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**Abstract:** *Panonychus ulmi* is widely distributed in apple and vineyards worldwide. In Brazil, the first damages were observed in apple orchard and later in grapevine. The aim of this study was to evaluate feeding preference and biological traits of *P. ulmi* on different grapevine varieties (BRS Vitória, Merlot (*Vitis vinifera*) and Concord (*Vitis labrusca*)) and apple Fuji (*Malus domestica*) under laboratory conditions. The food preference experiment did not reveal any significant differences, but *P. ulmi* presented the lowest rate of oviposition in Concord, demonstrating an oviposition preference in *V. vinifera* varieties and general preference for apple. Mortality was significantly different between varieties, with the lowest mortality on apple and higher oviposition rate in this host. Higher viability occurred on Merlot and apple. Feeding preference studies are scarce, considering phytophagous mites and grapevine varieties, although they are important in the development of pest control strategies.

**Keywords:** Host plant preference; Tetranychidae; *Vitis vinifera; Vitis labrusca.*

## INTRODUCTION

The two species of the genus *Panonychus* with economic interest, are *Panonychus ulmi* (Koch) and *Panonychus citri* (McGregor) (Acari: Tetranychidae) (Jeppson *et al.* 1975, Van Leeuwen *et al.* 2015). *Panonychus ulmi* reaches the level of economic damage in apple worldwide (Yin *et al.* 2013). In Brazil, the first record of *P. ulmi* occurred in apples from Argentina (Flechtmann 1967) and in 1972, Bleicher (1974) observed a large infestation in an apple orchard in Fraiburgo, Santa Catarina. The first record in grapevine in Brazil was carried out by Ferla and Botton (2008), in the Merlot variety in Bento Gonçalves, Serra Gaúcha, Rio Grande do Sul, in the 2005-2006 harvest. In 2006, the second record in *Vitis vinifera* was reported by Mendonça (2009), in Pirapora, Minas Gerais.

*Panonychus ulmi* was reported as an important pest in grapevines in Brazil since the first report (Ferla & Botton 2008). Its life cycle is characterized by egg, larva, protonymph, deutonymph and adult phases. At each stage, the mite passes through a period where it feeds intensely and after a resting period, followed by ecdysis. High temperatures and dry climate favor its reproduction and multiplication (Cuthbertson & Murchie 2005). This mite feeding causes loss of leaf color, and occasionally, a more uniform and tanned discoloration, which might lead to premature falling of leaves in high infestations, and a decrease in $CO_2$ exchange rates (Rilling & Düring 1990, Ferla & Botton 2008, Moraes & Flechtmann 2008, Duso *et al.* 2012). In the long term, these damages reduce accumulation of nutrients and the damages persist through the following season (Botha *et al.* 2005).

European grapevines (*Vitis vinifera* L.: Vitaceae) have the best characteristics for wine production (Souza 1996, Bouquet *et al.* 2006). Table grapes are also varieties of this same species, they are sensitive to fungal diseases and highly demanding regarding farming practices. They are very much appreciated for consumption in natura (Leão 2004). In Bento Gonçalves, Serra Gaúcha, Brazil outstanding varieties are BRS Vitória (as a table grape variety) and Chardonnay and Merlot (in wine production). American grapevines (*Vitis labrusca* L.: Vitaceae) are characterized by having high yield and being resistant to fungal diseases (Camargo & Maia 2008). Varieties of this species are usually used in juice production. In Serra Gaúcha, Concord and Niagara are the main varieties. Different varieties might show different susceptibility to pests and diseases, and it is important to know the level of resistance or susceptibility of the cultivated varieties for integrated pest management (Valadão *et al.* 2012).

Plant suitability as insect and mite host depends on factors such as its physiological condition, age, turgor, treatments with agrochemicals, and the environment (De Ponti 1977, Raupp *et al.* 2010). In artificial tests, the suitability of plants to mites is determined mostly by measuring reproduction 24, 48, or 72 hours after inoculation. Differences in host suitability are expressed by differences in longevity, oviposition, and mortality, resulting in differences in net mite reproduction (De Ponti 1977).

Studies to determine feeding preference, oviposition and viability of populations of phytophagous mites such as *P. ulmi* in different varieties of grapevine and apple in Rio Grande do Sul, Brazil are important, due to the lack of information. Considering field observations, *P. ulmi* is more prevalent in apple trees and in certain grape varieties in the Serra Gaúcha. Therefore, the authors raised the hypothesis that *P. ulmi* prefers to feed and will present different biological indices in apples, and that varieties of *V. vinifera* could be more susceptible to attack than *V. labrusca*. Additionally, the aim of the present study was to check the feeding preference of *P. ulmi* in the presence of apple leaves and different grapevine varieties, as well as to evaluate mortality, oviposition and viability, and duration of immature stages on leaves of these varieties.

## MATERIAL AND METHODS

### *Rearing stock*

Colonies were initiated with *Panonychus ulmi* specimens collected from grapevine leaves from *Vitis vinifera* variety Merlot from the 2016/17 harvest in Bento Gonçalves, (29°11'35.0" S, 51°34'53.3" W), State of Rio Grande do Sul, Brazil. Colonies were maintained in the laboratory on apple leaves *Malus domestica* Borkh. variety Fuji. The rearing was maintained for at least two generations on apple leaves, due to greater leaf durability compared to grapevine leaves, since this had to be replaced before the complete life cycle of the mite.

Leaves were placed with their abaxial face downwards on foam and moistened germinative paper inside plastic trays, with leaf edges covered with hydrophilic cotton. Distilled water was daily added to the tray to keep the cotton moistened. Populations were shifted from one arena to the other when leaves started to become yellowish, thus indicating they were inadequate for colony maintenance. To perform the experiments, females were dated, i.e., separated from the deutonymph phase in an arena, and after four to five days, they were used to perform the tests.

### *Experimental procedures*

This study was conducted at Laboratory of Acarology of Universidade do Vale do Taquari - Univates, in the 2016/2017 harvest. The specimens used in the experiment were obtained from rearing stocks maintained in the laboratory. The varieties used in the experiments were chosen according to the occurrence records of *P. ulmi* in the field.

### *Experiment 1: Feeding preference*

The feeding preference of *P. ulmi* was tested on *Vitis vinifera* varieties BRS Vitória and Merlot and in *V. labrusca* variety Concord, and apple *Malus domestica* variety Fuji. For this experiment, cage-shaped acrylic plates (35 x 80 mm) were used (Figure 1). Grapevine and apple leaf discs, in different combinations, were placed in two chambers (15 mm diameter and 3 mm) connected by a T-shaped maze on the crossbar (Ø 15 mm) and a lower circular chamber (Ø 5 mm) at the bottom end of the vertical bar of the T. This T, which connects the three chambers, has a 2-mm wide aisle. The lower part of the cage was closed with a fine mesh to allow for air exchange and a microscope slide was placed on the upper side (Schausberger & Hoffman 2008).

All adult females used in the experiment were kept starving for 12 hours and were individually placed in lower chambers to begin the experiments. Each specimen was transferred to leaf discs inside a chamber using a thin-tipped brush. When it was released, the specimen could choose between Concord X BRS Vitória; Fuji X Concord; BRS Vitória X Fuji Merlot X BRS Vitória; Merlot X Concord and Merlot X Fuji. Cages were checked every 1, 3, 7 and 24 hours after release, where the phytophagous mites were located. Experimental cages were kept in an acclimatized room at 25 ± 1 ºC, 65 ± 5 % RH, and 16:8 L:D photoperiod. A total of 20 replicates were performed for each substrate combination.

### *Experiment 2: Mortality, oviposition and viability of* P. ulmi *on different tested plants*

For the study of feeding preference of *P. ulmi*, arenas (5 cm diameter and 1.5 cm depth) were prepared containing leaf discs with distilled water-soaked cotton. Leaf discs clipped from BRS Vitória, Concord and Merlot varieties, and Fuji apple were placed onto these discs with their

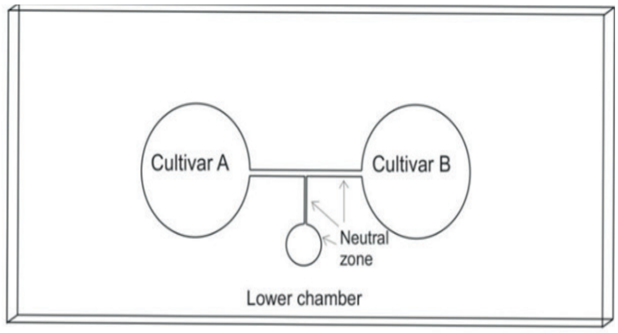


**Figure 1**. Cage-shaped acrylic plate used for the feeding choice test.

abaxial face downwards. Three adult *P. ulmi* females were released in each arena with the help of a thin-tipped brush.

Arenas were examined once a day (every 24 hours) during a period of five days. The number of live specimens and oviposition were recorded. On the fifth day, females that were still alive were removed and eggs were monitored until larvae hatching to observe viability. A total of 20 replicates were performed for each substrate. Arenas were kept in a climate chamber at 25 ± 1 ºC, 65 ± 5 % RH, and 16:8 L:D photoperiod.

### *Experiment 3: Suitability of tested plants varieties on immature development stages of* P. ulmi

To study suitability on *P. ulmi* development on different varieties, 28 arenas (5 cm diameter and 1.5 cm depth) were prepared for each variety. Distilled water-soaked cotton discs were placed on these arenas, and on top, leaf discs clipped from BRS Vitória, Merlot, Concord and apple were placed with the abaxial face downwards. Adult females of *P. ulmi* were transferred from the rearing stock to the arenas, and after a period of six hours, the rearing units were examined removing the females from those where oviposition had occurred, as well as surplus eggs, leaving only one egg per arena.

The remaining units were examined again after another period of six hours, repeating this process until a total of 28 eggs was obtained. The arenas were examined three times a day, at 7 a.m., 1 and 7 p.m., until mites reached the adult stage, to determine the developmental time of each mite. Mite populations were transferred to a new arena when leaves started to become yellowish, thus indicating they were inadequate for colony maintenance. Arenas were kept in an acclimatized room at 25 ± 1 ºC, 65 ± 5 % RH, and 16:8 L:D photoperiod.

### *Statistical analysis*

In order to determine the differences in the frequency of occurrence of *P. ulmi* individuals on different grapevine varieties and on apple of Fuji variety at different moments, a binomial statistical test of two proportions was used. When there was no choice for either treatment evaluated, the individual was considered to be in the neutral zone, and was not considered in the analysis. The Kruskal-Wallis test was used, with an aposteriori Dunn test, at a 5 % significance level to test mortality, oviposition, and viability among the varieties tested. An ANOVA test followed by Tukey's test were performed to test the development stages of *P. ulmi* on different leaf types tested. Bioestat 5.3 was the statistical program used to analyze the results (Ayres *et al.* 2007).

## RESULTS

### *Experiment 1*

There was no significant difference in the feeding preference of *P. ulmi* between Merlot X Concord and Merlot X apple at any of the times observed. However, when released with a chance of choosing between Concord X BRS Vitória, *P. ulmi* preferred Concord ($Z = 2.54$; $p = 0.01$) at 24 hours when (Figure 2B) apple X Concord were offered, there was preference for Concord at 1h ($Z = -4.15$; $p = 0.0001$) and for apple at 24 h ($Z = 2.92$; $p = 0.03$) (Figure 2A); in the presence of BRS Vitória X apple this mite preferred apple at 24 h ($Z = 2.92$; $p = 0.03$); between Merlot X BRS Vitória, it preferred BRS Vitória at 1 h ($Z = -1.77$; $p = 0.02$) and 3 h ($Z = -3.31$; $p = 0.0009$) (Figure 2A and B). In the remaining evaluations, no significant difference was observed between varieties (Figure 2 A; B and C).

### *Experiment 2*

There was significant difference in mortality on the different varieties tested ($H = 29.51$; $p < 0.01$). lower mortality rate was observed on apple, with mean mortalities of 9.3 ± 0.53. There were intermediate mortality values on BRS Vitória (30.66 ± 1.02), Concord (25.66 ± 0.65), and Merlot varieties (22.33 ± 0.85) (Figure 3). The highest viability occurred on Merlot (95.26 ± 3.91) and apple (89.10 ± 5.14) ($H = 189.04$; $p < 0.01$) (Figure 3). There was significant difference in oviposition between the varieties tested ($H = 206.36$; $p < 0.01$). The highest oviposition rate occurred on apple (11.84 ± 5.43), followed by Merlot (5.49 ± 4.43) and BRS Vitória (4.88 ± 3.66). The lowest oviposition occurred on Concord (1.11 ± 1.43) (Figure 4).

### *Experiment 3*

The results obtained showed that *P. ulmi* developed on all varieties tested in this study (Table 1). In variety Concord, development speed was slower lower, protochrysalid and egg-adult stages of females and males lasted longer, compared to the other varieties. The egg-adult period on apple, BRS Vitória, Merlot and Concord was, on average, 11.81, 12.18, 11.11, and 13.91, respectively. (Table 1). In addition, the deutonymph stage was longer than the others on BRS Vitória. In all other situations, there were no significant differences between the *P. ulmi* host varieties.

## DISCUSSION

In the present study, *P. ulmi* showed preference for apple leaves through the biological indices evaluated in the experiments, corroborating our first hypothesis. Native of Europe, *P. ulmi* is widely distributed worldwide (Jeppson *et al.* 1975, Van Leeuwen *et al.* 2015), but the first record on grapevines in Brazil occurred about 40 years later (Ferla & Botton 2008) than the first record in apple orchards (Flechtmann 1967, Bleicher 1974). Therefore, the preference for apple as host might be explained by the fact that this mite species is more adapted to this plant than to the different grapevine varieties.

*Panonychus ulmi* preferred *V. vinifera* as host instead of *V. labrusca* at the end of the experiment, with lowest oviposition was observed in the Concord variety. Stimuli emitted by the plant induce the mite to oviposit or feed (Lara 1991). Therefore, *P. ulmi* has a non-preference and/or to *V. labrusca* since this was the less frequently used species for oviposition and the viability was also lower than in the other varieties.

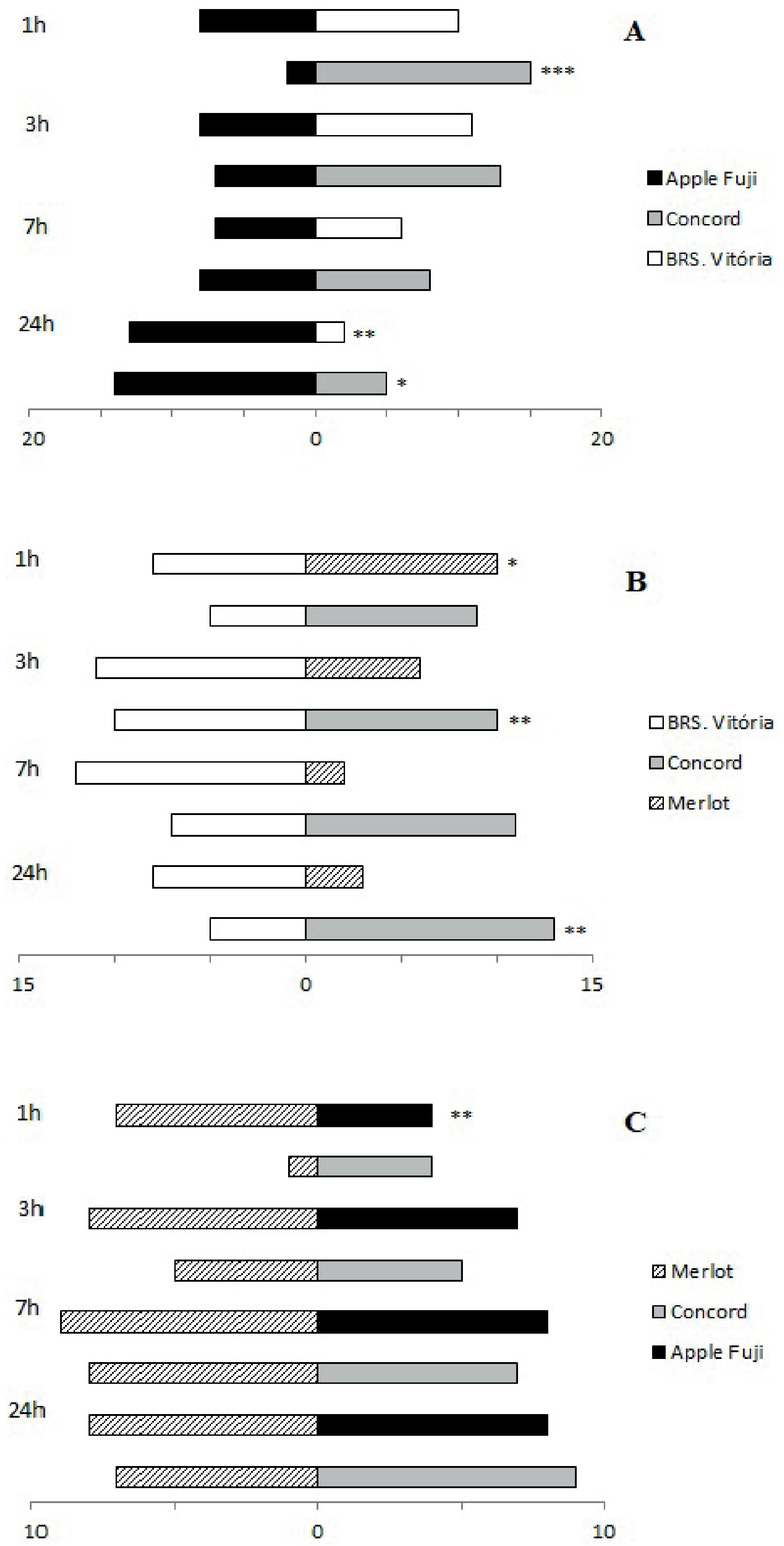


**Figure 2.** Food preference of *Panonychus ulmi* for grapevine varieties or apple Fuji variety after 1, 3, 7 and 24 hours at 25 ± 1 °C, 65 ± 5% RH, and 16:8 L:D photoperiod. Binomial test in two proportions at significance levels of: ***p < 0.0001; **p < 0.01; *p < 0.05.

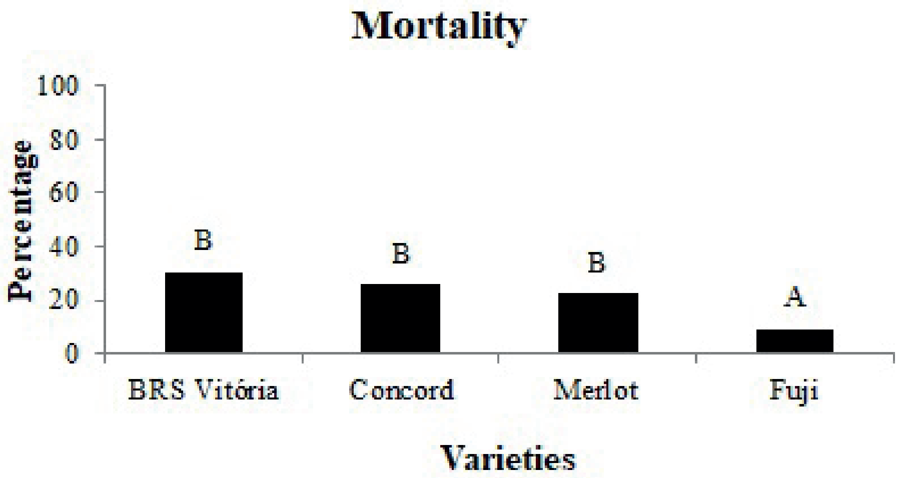


**Figure 3**. Mortality (%) of *Panonychus ulmi* on apple (Fuji) and grapevines (BRS Vitória, Merlot and Concord) maintained for a period of five days at 25 ± 1°C, 65 ± 5% RH, and 16:8 L:D*.*Percentages followed by the same letter do not differ using the Kruskal-Wallis test, with a posteriori Dunn test, at 5% of probability.

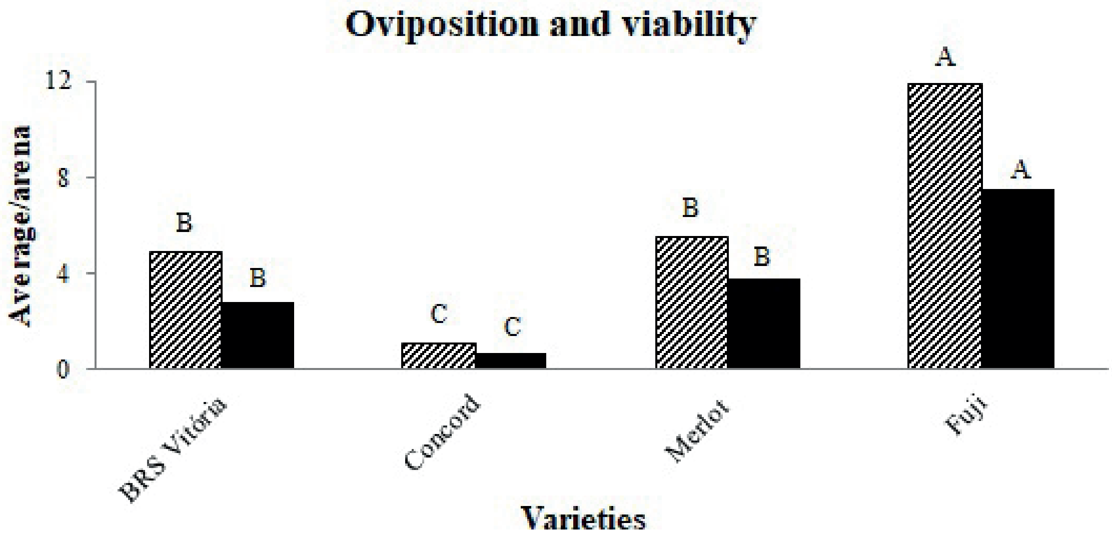


**Figure 4**. Average oviposition and viability of *Panonychus ulmi* maintained on apple (Fuji) and grapevines (BRS Vitória, Merlot and Concord) at 25 ± 1°C, 65 ± 5% RH, and 16:8 L:D.*Averages followed by the same letter do not differ using the Kruskal-Wallis test, with a posteriori Dunn test, at 5% of probability.

Differential responses from phytophagous mites might vary according to the quality of the host plant, depending also on the quantity and nature of primary and secondary metabolites (Awmack & Leather 2002, Boom *et al.* 2003). These factors might vary among plant species and even between varieties (Vásquez *et al.* 2008). These results are reinforced since grapevine varieties derived from crossing with *V. labrusca* were observed to have a lower level of damage caused by *Popillia japonica* Newman (Coleoptera: Scarabaeidae). Therefore, *V. labrusca* is considered more resistant than *V. vinifera* (Gu & Pomper 2008). Some plants might develop their self-protection strategies modifying morphological structures e.g. trichomes, thorns, cell wall structures, depositing silica, and forming inorganic crystals, among others (Lucas *et al.* 2000). These changes occur for the plant to protect itself from physical and biological threats, preventing the herbivore from feeding, by causing damages to the buccal apparatus (Lucas *et al.* 2000). This process is continuous, and possibly, the herbivore will develop strategies to overcome these defense changes of the plant.

**Table 1.** Mean (± SE) developmental time and adult longevity (days) of *Panonychus ulmi* feeding on Apple of Fuji variety and grapevines of BRS Vitória, Merlot and Concord varieties, under maintained in a climate chamber at 25 ± 1 °C, with a 12 hours photoperiod, and 80 ± 5 % RH. *Values followed by the same letters do not significantly differ in the column regarding each parameter ($P < 0.05$, ANOVA followed by post-hoc Tukey tests).

| Host | Egg | Larva | Protochrysalid | Protonymph | Deutochrysalid | Deutonymph | Teliochrysalid | Egg-adult period | Egg-adult period - Female | Egg-adult period - Male |
|---|---|---|---|---|---|---|---|---|---|---|
| Fuji | 5.9 ± 0.15a* | 0.82 ± 0.11a | 0.51 ± 0.05b | 0.82 ± 0.06a | 0.64 ± 0.06a | 0.66 ± 0.07b | 0.74 ± 0.06a | 11.81 ± 0.25b | 11.96 ± 0.45ab | 11.64 ± 0.22b |
| BRS Vitória | 5.89 ± 0.15a | 0.90 ± 0.08a | 0.60 ± 0.04b | 0.78 ± 0.06a | 0.62 ± 0.07a | 1.09 ± 0.11a | 0.76 ± 0.06a | 12.18 ± 0.21b | 12.25 ± 0.26ab | 12.00 ± 0.40b |
| Merlot | 5.71 ± 0.16a | 0.66 ± 0.07a | 0.48 ± 0.04b | 0.69 ± 0.10a | 0.52 ± 0.05a | 0.68 ± 0.10b | 0.66 ± 0.09a | 11.11 ± 0.31b | 10.70 ± 0.38b | 11.33 ± 0.43b |
| Concord | 5.86 ± 0.12a | 0.92 ± 0.11a | 0.80 ± 0.06a | 0.88 ± 0.14a | 0.72 ± 0.08a | 1.38 ± 0.25a | 0.88 ± 0.19a | 13.91 ± 0.74a | 14.13 ± 1.13a | 13.83 ± 0.96a |

Considering morphology, is possible to observe trichomes in grapevine leaves. *Vitis labrusca* recognizably shows high density of trichomes on the abaxial face, while these structures have lower density in *V. vinifera* (Kortekamp & Zyprian 1999). This factor could discourage the biting or oviposition by plant predators (Chapman 1977). Moreover, the density of foliar trichomes might affect the movement, feeding, and oviposition of mites, as was observed for *Polyphagotarsonemus latus* Banks on pepper (*Capsicum praetermissun* Heiser and P. G. Smith; Solanaceae) (Matos *et al.* 2009). This non-preference for *V. labrusca* was also observed in a study conducted by Valadão *et al.* (2012) with Niagara Rosada variety, when *Tetranychus urticae* Koch showed lower fecundity and survivorship. The authors also noticed that there was higher fleeing intensity from this variety, thus suggesting a resistance mechanism by non-preference. Both our experiments made it evident that *P. ulmi* has feeding preference for apple and *V. vinifera* varieties. The impact of leaves of different varieties on mite development stages, which was low on *V. labrusca*, was also evident.

Fecundity of *Tetranychus cinnabarinus* (Boisduval) varied from 19 to 400 eggs in Cucurbita and Lagenaria (*Lagenaria siceraria* (Molina) Standl.: Cucurbitaceae) pumpkin varieties (Edelstein *et al.* 2000) whereas *T. urticae* had higher populations in Hapil and Pegasus (*Fragaria* x *ananassa* Duchesne ex Rozier: Rosaceae) strawberry varieties than in the others (Sonneveld *et al.* 1996). In addition, Bailey *et al.* (1978) working with cotton (*Gossypium hirsutum* L.: Malvaceae), observed that morpho-anatomical characteristics such as trichomes and leaf glandules, and the thickness of the epidermal-cuticle layer might comprise physical barriers for tetranychid feeding.

The results of the present study indicate that *V. vinifera* varieties are similarly suitable as *P. ulmi* hosts, as the same phytophagous mite species was observed by Klock *et al.* (2011) in Rio Grande do Sul. However, Johann *et al.* (2019) observed the development of *P. ulmi* was similar in both grapevine cultivars evaluated as Cabernet Suvignon and Pinot Noir, although *V. vinifera*, when its prosthesis was smaller than apples, however, the reproductive potential in vines is lower. These studies reinforcing the results of our study an arrangement of *P. ulmi* by apple and *V. vinifera* vines. The physical characteristics of leaves might determine this preference and might also influence the development of the biological cycle of the mite. Therefore, *V. vinifera*, with thinner, soft leaves, and lower number of trichomes, would be more appreciated for oviposition, while *V. labrusca*, with thicker leaves, higher abundance of trichomes, epidermis with more lignin and wax, would be recognized as unsuitable for the development of *P. ulmi* offspring and population.

Feeding preference studies are scarce despite their importance in determining if a phytophagous mite species has infestation potential for a given variety or if the plant actually has a property that repels this mite, thus inhibiting its presence and egg laying. Identifying grapevine varieties that are not preferred by phytophagous mites is important in the development of pest control strategies that involve decreased use of chemical products. We can conclude that *P. ulmi* showed different preference levels for the leaves of the grapevine varieties offered. Apple was preferred over grapevine varieties, and *V. vinifera* varieties were preferred over those of *V. labrusca*. In order to confirm these results, the authors recommend further studies on *P. ulmi* biology with the grapevine varieties evaluated in this study.

## ACKNOWLEDGMENTS

This study was partly financed by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001. Noeli Juarez Ferla (311307/2014-0) is supported by a CNPq productivity research grant. We also thank the two anonymous reviewers for improving the manuscript.

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*Submitted: 1 April 2020*
*Accepted: 26 August 2020*
*Published on line: 11 September 2020*
*Associate Editor: Gudryan Barônio*