

Scientific Note

Oviposition of *Panonychus ulmi* (Koch, 1936) (Acari: Tetranychidae) in response of conspecific and heterospecific mites cues

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Abstract. This study aimed to assess oviposition of *Panonychus ulmi* (Koch, 1936) (Acari: Tetranychidae) in response of conspecific and heterospecific mites cues on apple trees (*Malus domestica* Borkh: Rosaceae). The oviposition of *P. ulmi* was measured on apple tree leaves with the presence of webs, eggs, and other cues of phytophagous mites (*Aculus schlechtendali* (Nalepa, 1890) (Acari: Eriophyidae), *P. ulmi*, *Tetranychus urticae* Koch, 1936 (Acari: Tetranychidae), and *Tetranychus ludeni* Zacher, 1913 (Acari: Tetranychidae)). The presence of conspecific webs and cues increased the oviposition of *P. ulmi*, while the presence of *A. schlechtendali* and *T. urticae* webs and eggs and *A. schlechtendali* cues decreased oviposition. The oviposition increases in the presence of conspecifics suggesting that *P. ulmi* performs better in environments without heterospecific mites. On the other hand, the *P. ulmi* decreases in oviposition in presence of heterospecific mite cues suggesting a potential competitive displacement of tetranychid by eriophyid mites.

Keywords: Phytophagous mites, interaction, *Malus domestica*.

Brazil is among the largest apples (*Malus domestica* Borkh: Rosaceae) producers in the world (Kist 2019; Kist et al. 2022). Brazilian production is primarily located in the higher regions of Southern Region (Kist 2019). Due to the expansion of cultivation areas it is observed a geographical distribution of agricultural pests (Wheeler & Hoebcke 2009). Furthermore, the continuous use of pesticides in orchards has led to a reduction in natural enemies and an increased phytophagous mites attack severity (Lorenzato & Secchi 1993; Walker et al. 2017; Schmidt-Jeffries & Beer 2018). Among the main mites species causing damage to apple trees worldwide are *Panonychus ulmi* (Koch, 1836), *Tetranychus urticae* Koch, 1836 (Acari: Tetranychidae), and *Aculus schlechtendali* (Nalepa, 1890) (Acari: Eriophyidae) (Jeppson et al. 1975; Ferla & Moraes 1998; Monteiro 2001; 2002; Nascimento et al. 2020; Kasap & Atlihan 2021).

Panonychus ulmi has been cited as the main phytophagous mite affecting apple orchards worldwide and it has been reported with populations exceeding the economic damage threshold level (Lorenzato et al. 1986; 1987; Ferla & Botton 2008; Yin et al. 2013; Kasap et al. 2019; Ivanović et al. 2022; Moraes et al. 2024). *Tetranychus urticae* has been reported causing damage around the world in apple cultivation (He et al. 2001; Liu et al. 2006; Funayama 2010; Kamusiime et al. 2023). In Brazil, *T. urticae* has been reported in apple orchards (Moraes et al. 2024). However, recent surveys have not found that species in the assessed orchards (Silva et al. 2022), or found small populations (Rode et al. 2023b). *Tetranychus ludeni* Zacher, 1913 (Acari: Tetranychidae) was recorded in apple tree orchard (Flechtmann 1996) and occurring and causing injuries in seedlings (Rode et al. 2023a). *Aculus schlechtendali* is considered of quarantine importance and recently reported in Brazil. It has been observed throughout the Southern Region of Brazil. Although, there are no records of damage in orchards yet (Ferla et al. 2018; Nascimento et al. 2020).

The presence of several species, each with their own survival strategies, in the same environment causes various positive and negative interactions (Fisher et al. 2021). The web produced by certain species of tetranychidae exerts effects on the environment, such as protecting the mites from environmental adversities and the presence of other species, both predators and phytophagous (Gerson 1985;

Moraes et al. 2024).

To date, there is limited information about the behavior of *P. ulmi* in the presence of other phytophagous mite species sharing the same environment. Thus, this study aimed to assess *P. ulmi* oviposition in response to conspecific and heterospecific mites cues on apple trees.

Apple seedlings were obtained from a commercial nursery grown in pots (11 L) filled with Carolina Soil® substrate and soil. Seedlings were kept in a protected area, and irrigated every two days. These plants were used for maintaining the arenas of mite stock cultures and for bioassays.

The stock culture of *A. schlechtendali* was established using specimens collected from apple trees. Arenas were maintained in rectangular trays (30 × 20 cm) containing moistened foam covered with germination paper. Apple tree leaves with an abaxial side facing up were placed on the paper. The leaves, edges and part of petiole were covered with moistened hydrophilic cotton. The *P. ulmi* collections were made in an apple orchard, and they were transferred to apple tree leaves arenas. These arenas were constructed similarly to the *A. schlechtendali* stock rearing, however with adaxial side facing upwards. The rearings were kept in laboratory conditions (25 ± 1°C, 14-hour photoperiod and relative air humidity (RH) of 75 ± 5%).

Rearings of *T. ludeni* were established using specimens collected from soybean (*Glycine max* (L.) Merrill: Fabaceae). The specimens were transferred to bean (*Phaseolus vulgaris* (L.): Fabaceae) cultivated in trays of 45 × 30 × 8 cm (7 L) with Carolina Soil® substrate. Rearings of *T. urticae* were pre-established in the Laboratory of Acarology at Univates (Labacari) and multiplied in bean plants similarly to rearings of *T. ludeni*. Infested bean trays were daily irrigated and kept in laboratory conditions.

To assess *P. ulmi* oviposition on apple trees a methodology was adapted from Sarmiento et al. (2011). Forty arenas were constructed from apple tree leaves for each of the mite species tested. Twenty ones for testing the influence of webs and eggs on the presence of *P. ulmi* and another twenty to evaluate the influence of the presence of other cues from the presence of mites species. The arenas were built in Petri dishes with moistened cotton on the bottom and edges of the leaves to prevent mites from escaping. On the cotton, an apple leaf

was placed with adaxial side facing up. The arenas for *A. schlechtendali* were constructed with abaxial side of the leaf facing up. We tested *P. ulmi* oviposition rates on leaves infested by conspecific (i.e., *P. ulmi*), by heterospecific (i.e., *T. urticae*, *A. schlechtendali*, *T. ludeni*) and without mites arena (i.e., control). Each arena received 10 mated females of conspecific or heterospecific mites, where they remained for two days feeding, ovipositing, defecating, and producing webs.

Effects of web and eggs. After two days of infestation, mites were removed from the arenas with the aid of a fine-tipped brush, taking care not to damage the web, and the web and eggs were kept for evaluation. In *A. schlechtendali* arenas, specimens were kept since this species does not produce webs, and visualizing their eggs was not possible. A dated and mated female of *P. ulmi* was released and kept for seven days in arenas. Oviposition was evaluated daily at 1:00 pm.

Effect of other cues. After two days of infestation, mites, eggs and webs were removed from the arenas. However, feces remained on the leaf surface. Subsequently, a dated and mated female of *P. ulmi* was released into each arena. The *P. ulmi* female was kept in the arenas for seven days with the presence of conspecific and heterospecific tracks, and daily oviposition assessment was conducted at 1:00 pm.

The arenas were kept in rectangular trays in laboratory conditions. The data were subjected to the Kruskal-Wallis non-parametric test followed by the Dunn's a posteriori test by BioEstat 5.3 (Ayres et al. 2007).

The presence of conspecific webs and eggs and those of *T. ludeni* caused an increase in the oviposition of *P. ulmi*, with no significant difference between them (Fig. 1A). The effect of *T. ludeni* on oviposition was similar to the control. The presence of *A. schlechtendali* eggs and webs and eggs of *T. urticae* decreased in *P. ulmi* oviposition.

Regarding the effect of other cues on the oviposition of *P. ulmi*, the conspecific cues caused a significant increase in oviposition, differing from the others (Fig. 1B). The presence of other cues from *T. urticae* and *T. ludeni* showed a response similar to the control. On the other hand, previous cues from *A. schlechtendali* caused a significant decline in *P. ulmi* oviposition.

The previous presence of conspecific may have favored oviposition and establishment of new populations, as the perception of conspecifics could be an indicator of safety in terms of the absence of natural enemies or other risks (Shiojiri et al. 2002; Rodriguez-Saona et al. 2005; Rodriguez-Saona & Thaler 2005). Given that some herbivores vary their oviposition based on interspecific competition, the pre-existence of the species at the site may suggest less competition for resources (Mayhew 1997; Gripenberg et al. 2010). Thus, new populations of *P. ulmi* may have perceived the location as a favorable site for establishment of their offspring as a survival strategy.

The decreased of oviposition of *P. ulmi* on apple leaves by presence of *T. urticae* (web and egg) may suggest interspecific competition (Ferragut et al. 2013; López-Olmos & Ferragut 2023) or even, the presence of *T. urticae* web could be harming the establishment of *P. ulmi*

on leaves (Moraes et al. 2024). Moreover, the decrease in oviposition of *P. ulmi* in presence of other cues of *A. schlechtendali*, and the mite itself may suggest a competitive displacement of the tetranychid due to the presence of the eriophyid, as observed by Croft & Hoying (1977). There are reports of competitive displacement due to the coexistence of species that use and compete for same spaces and resources (López-Olmos & Ferragut 2023). Although competition is one of the regulators of species abundance within a community, and despite its economic importance, few studies address competition among mites (Reitz & Trumble 2002). Therefore, specific studies on competition among mite species at apple orchards are recommended.

In addition to the direct influence, exerted in certain situations by web (Moraes et al. 2024), differential oviposition responses, both in terms of conspecific perception and potential competition with heterospecifics, may be related to the chemical substances released by the species, such as kairomones and infochemicals, which are responsible for information transmission (Dicke & Sabelis 1988; Dicke et al. 1990). Similar to many studies demonstrating the influence of volatile released by plants on predator-prey behavior in a tritrophic context, these kairomones can be compounds associated with herbivores themselves present in their feces, eggs, exuviae, webs, and pheromones (Collier et al. 2000; Afsheen et al. 2008; Peñaflores 2019), mediating the behavior and interaction among organisms (Collier et al. 2000; Ayelo et al. 2021).

Studies like this are essential as they enable the understanding of the interaction of the species of phytophagous mites on apple trees with the environment and other species, assisting in decision-making regarding the most appropriate management practices for cultivation.

It can conclude that the previous presence of conspecifics increases the oviposition of *P. ulmi* on apple leaves, while the presence of heterospecifics decreases *P. ulmi* oviposition, indicating that the species performs is better at sites without heterospecifics and where conspecifics have been and demonstrated safety for the development. And the decrease in oviposition of *P. ulmi* in presence of *A. schlechtendali* and *T. urticae*, suggest competitive displacement of tetranychid by the eriophyid mites and difficulty in establishing *P. ulmi* in presence of tetranychid, with high web production. These results provide a foundation for further research related to interaction among phytophagous mites and their predators on apple trees.

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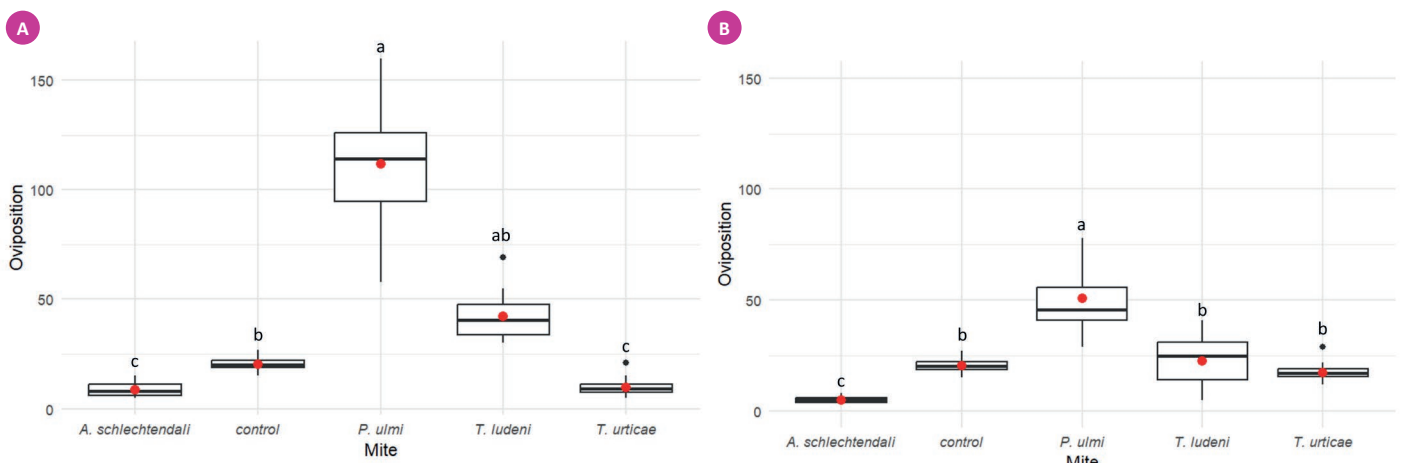


Figure 1. Average oviposition rate of *Panonychus ulmi* on apple leaf discs with injury, web and eggs (A) and cues (B) of *Aculus schlechtendali*, *Panonychus ulmi*, *Tetranychus ludeni*, *Tetranychus urticae* and control, over a period of seven days. Different letters denote significant differences between treatments using the Kruskal-Wallis test, followed by Dunn's test ($\alpha = 0.05$).

Authors' Contributions

PAR: Conceptualization, Data curation, Formal analysis, Investigation and Methodology, Validation and visualization, Writing - original draft, Writing - review & editing. JRS: Data curation, Formal analysis, Writing - review & editing. NJF: Conceptualization, Funding acquisition, Project administration, Software, Resources and Supervision, Writing - review & editing.

Conflict of Interest Statement

The authors declare no conflict of interest.

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