

Behavior and injuries of phytophagous mites on apple trees (*Malus domestica*: Rosaceae)

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Abstract

This study aimed to assess the behavior of phytophagous mites, including conspecific and heterospecific effects, and the injuries caused by them at different population levels on apple tree leaves (*Malus domestica*: Rosaceae). The oviposition of *Panonychus ulmi* (Koch) was evaluated on apple tree leaves with the presence of webs, eggs, and cues of phytophagous mites (*Aculus schlechtendali* (Nalepa), *P. ulmi*, *Tetranychus urticae* Koch, and *Tetranychus ludei* Zacher). The response to different infestation levels of *P. ulmi*, *T. urticae*, and *T. ludei* on apple tree leaves was assessed through chlorophyll A, B, and total chlorophyll indices and membrane stability loss. 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 decreased oviposition. The presence of *A. schlechtendali* cues also led to a decline in *P. ulmi* oviposition. Leaves infested with *T. urticae* showed higher chlorophyll loss, leaves infested by *P. ulmi* and *T. ludei* had decreased chlorophyll averages in treatments with 25 mites/leaf and above. The evaluation of membrane stability loss identified *T. ludei* as a potential cause of injuries in apple trees. The evaluations indicated that the higher the number of mites on the leaf surface, the larger the damaged area. This work warning to the apple production chain for monitoring infestations and preventing a potential threat to culture. These results provide a foundation for further research on the potential of apple tree phytophagous mites and their interactions, both among herbivores and with predators.

Introduction

Brazil is among the largest producers of apples (*Malus domestica* Borkh: Rosaceae) in the world, and the quality of the fruits in the country has been increasing each year (Kist et al. 2019, 2022). Brazilian production is primarily located in the higher regions of the three southern states, due to the favorable climate for cultivation (Kist et al. 2019). However, the expansion of cultivation areas has contributed to the geographical distribution of agricultural pests that attack the crop (Wheeler and Hoebeke 2009). Furthermore, the continuous and indiscriminate use of pesticides in orchards has led to a reduction in natural enemies and an increase in pests, such as phytophagous mites (Lorenzato and Secchi 1993; Walker et al. 2017; Schmidt-Jeffries and Beer 2018), potentially leading to species resistance, further complicating control efforts (Bajda et al. 2015; Rameshgar et al. 2019; Mota-Sanchez and Wise 2023).

When populations reach pest status in the crop, they cause environmental, economic, and social impacts (Brown and Hovmøller 2002; Anderson et al. 2004; Navia et al. 2011). Among the main species causing damage to apple trees worldwide are *Panonychus ulmi* (Koch), *Tetranychus urticae* Koch (Tetranychidae), and *Aculus schlechtendali* (Nalepa) (Eriophyidae) (Jeppson et al. 1975; Ferla and Moraes 1998; Monteiro 2001, 2002; Nascimento et al. 2020; Kasap and Atlihan 2021).

Panonychus ulmi has been reported with populations exceeding the economic damage threshold in apple orchards worldwide, and for decades, it has been cited as the main phytophagous mite affecting the crop (Lorenzato et al. 1986, 1987; Moraes and Flechtmann 2008; Ferla and Botton 2008; Yin et al. 2013; Kasap et al. 2019; Ivanović et al. 2022). The damage caused by this species is characterized by leaf bronzing, and in severe infestations, it can lead to premature leaf drop (Moraes and Flechtmann 2008). The species has a high dispersal capability and an aptitude for colonizing new areas due to the occurrence of winter eggs, high reproductive rates, and a reproductive mode that allows a small number or even a single female to initiate a rapidly growing colony (Helle and Sabelis 1985).

Tetranychus urticae, considered economically significant in various crops, including fruit trees, vegetables, and flowers, is a major pest in greenhouses, cultivated fields, and orchards (Bolland et al. 1998; Van Leeuwen et al. 2010). It disperses through the air over long distances while migrating shorter distances among nearby trees in apple

orchards (Uesugi et al. 2009). In apple cultivation, it has been reported causing damage in various parts of the world (He et al. 2001; Liu et al. 2006; Funayama 2010; Kamusiime et al. 2023). In Brazil, it has been reported in apple orchards (Moraes and Flechtmann 2008) however, recent surveys have not found the species in the assessed orchards (Silva et al. 2022).

Aculus schlechtendali, considered of quarantine importance and recently reported in Brazil, has been observed throughout the southern region of the country, although there are no records of damage in Brazilian orchards yet (Ferla et al. 2018; Nascimento et al. 2020). In countries where the species is considered economically significant, the damage is attributed to the piercing of plant epidermal cells by its stylets, resulting in symptoms characterized by bronzing on the surface of leaves, flowers, and fruits (Duso et al. 2010). The presence of *A. schlechtendali* raises concerns about the potential for this species to reach pest status if not controlled early, becoming a problem for farmers (Nascimento et al. 2020; Corrêa et al. 2021).

Tetranychus ludeni Zacher (Tetranychidae) is associated with over 250 species of host plants, including some of economic importance (Zhang 2002; Adango et al. 2006; Moraes and Flechtmann 2008; Gent et al. 2009; Reichert et al. 2014; Gotoh et al. 2015; Fathipour and Maleknia 2016). The species tends to occur in high populations on the adaxial surface of the apical and median leaves of plants, primarily in cotton and soybean crops, causing injuries and damage (Zhang 2002; Adango et al. 2006; Moraes and Flechtmann 2008; Reichert et al. 2014; Gotoh et al. 2015; Toldi et al. 2016). In apple trees, there is a record of *T. ludeni* occurrence in an orchard (Flechtmann 1996) and another occurring and causing injuries in seedlings (Rode et al. 2023), serving as a warning to the cultivation due to the potential for future infestations in orchards and damage to plants.

Infestations of phytophagous mites pose problems as they cause injuries and result in the loss of leaf and fruit quality (Flechtmann 1975; Ochoa 1991). The damage caused by these organisms is primarily due to direct effects, involving the destruction of leaf parenchyma and an increase in transpiration from the attacked leaf (Moraes and Flechtmann 1981). Tetranychids pierce plant epidermal cells to feed, causing leakage of cytoplasmic content, air entry, increased transpiration due to water deficit, and changes in coloration due to chlorophyll removal, resulting in a decrease in the plant's photosynthetic capacity (Moraes and Flechtmann 2008; Vacante 2015). Chlorophylls are the most abundant natural pigments present in plant leaves, contributing to the absorption of photosynthetically active radiation and being crucial for oxygen and sugar production (Blackburn 2007; Jay et al. 2017). Chlorophyll content is an important indicator of the physiological state of plants and is widely used to analyze the development and growth of the plant under study (Blackburn 2007; Steele et al. 2008; Sperotto 2014). Additionally, monitoring chlorophyll loss in plant tissues is one of the indicators that allows the determination of a plant's tolerance to phytophagous pest attacks (Smith 2005), assisting in orchard management and indicating fruit quality.

To date, there is limited information about injuries caused by phytophagous mites in apple trees in Brazil, especially regarding the potential damage caused by *T. ludeni* to the crop. Additionally, little is known about the behavior of *P. ulmi* in the presence of other phytophagous species sharing the same environment. Thus, this study aimed to evaluate the behavior of phytophagous mites, including conspecific and heterospecific effects, and the injuries caused by them at different population levels on apple tree leaves.

Materials and methods

Apple trees

Seedlings of the Eva apple cultivar were obtained from a commercial nursery in the city of Lapa, state of Paraná. The plants were grown in black plastic pots (11L) filled with Carolina Soil® substrate and soil, kept in a protected area, and irrigated every two days. These plants were used for maintaining the arenas of phytophagous mite stock cultures and for the bioassays conducted during the study period.

Phytophagous mite stock cultures

Aculus schlechtendali. The stock culture was established using specimens collected from apple tree leaves in the municipality of Antônio Prado, state of Rio Grande do Sul (28°53'23"S 51°23'06"W). The arenas were maintained in rectangular trays (30 × 20 cm) containing moistened foam covered with germination paper. Two apple tree leaves with the abaxial side facing up were placed on the paper, and the edges and part of the petiole were covered with moistened hydrophilic cotton. Each arena received 30 specimens. A rectangular glass was placed over each tray to maintain humidity in the arenas. The arenas were kept in a climate-controlled room at 25 ± 1°C, with a 14-hour photoperiod and relative air humidity (RH) of 75 ± 5%. Daily, the arenas were moistened to maintain the vigor of the apple tree leaves. New arenas were constructed whenever the leaves showed a yellowish color, indicating they were unsuitable for maintaining mite populations.

Panonychus ulmi. For the establishment of cultures, collections were made in an apple orchard in the municipality of Antônio Prado. The methodology adopted for the cultures was adapted from Herbert (1981). Mites were transferred to arenas made with apple tree leaves. The arenas were constructed in rectangular trays (30 × 20 cm) containing moistened foam covered with germination paper. Two apple tree leaves with the adaxial side facing up were placed on the paper, and the edges and part of the petiole were covered with moistened hydrophilic cotton. Each arena received 20 adult females of *P. ulmi* and was kept in a germination chamber at 25 ± 1°C, with a 14-hour photoperiod and RH of 75 ± 5%, receiving daily irrigation to maintain leaf vigor.

Tetranychus ludeni. Cultures were established using specimens collected from soybean plants (*Glycine max* (L.) Merrill: Fabaceae) in the city of Muitos Capões, RS (28°21'32.4"S 51°14'46.2"W). The specimens were transferred to bean plants (*Phaseolus vulgaris* (L.): Fabaceae) cultivated in trays of 45 × 30 × 8 cm (7 L) with Carolina Soil® substrate. Infested bean trays were kept in a germination chamber at 25 ± 1°C, with a 14-hour photoperiod and RH of 75 ± 5%, receiving daily irrigation to maintain leaf vigor.

Tetranychus urticae. Cultures pre-established in the Laboratory of Acarology at Univates (Labacari) were multiplied. The specimens were transferred to bean plants cultivated in trays of 45 × 30 × 8 cm (7 L) with Carolina Soil® substrate.

Oviposition of *Panonychus ulmi*

Species combinations. The methodology for assessing the effect of damage, webbing, and the presence of other phytophagous mite species on the oviposition of *P. ulmi* was adapted from Sarmiento et al. (2011). Forty arenas were constructed from apple tree leaves for each interaction of mite species, with 20 for testing the influence of webs and eggs on the presence of *P. ulmi* and another 20 to evaluate the influence of the presence of cues on the presence of the species. The arenas were constructed in Petri dishes with the bottom covered with a circle of cotton moistened with distilled water. On the cotton, an apple tree leaf was placed with the adaxial side facing up. The arenas for *A. schlechtendali* were constructed with the abaxial side of the leaf facing up, keeping the cotton moistened at the edges to prevent mite escape. The tested mite interactions were: *P. ulmi* × *P. ulmi*; *P. ulmi* × *T. urticae*; *P. ulmi* × *A. schlechtendali*; *P. ulmi* × *T. ludeni*; *P. ulmi* × Control (without the presence of another species in the arena). Each arena

received 10 mated females of conspecific or heterospecific mites, where they remained for two days feeding, ovipositing, and producing webs.

Effects on conspecifics and heterospecifics. After two days of infestation, mites from different species 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, the specimens were kept as 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 four days in each arena. Oviposition was evaluated daily at 1:00 pm. The arenas were kept in rectangular trays at $25 \pm 1^\circ\text{C}$, with a 14-hour photoperiod and RH of $75 \pm 5\%$. The data were subjected to the Kruskal-Wallis test followed by the Dunn test, using BioEstat 5.3 software at a significance level of 5% (Ayres et al. 2007).

Effect of olfactory cues on conspecifics and heterospecifics. After two days of infestation, mites were removed from the arenas along with eggs and webs. Subsequently, a dated and mated female of *P. ulmi* was released into each arena. The *P. ulmi* female was kept in the arenas for four days, and daily oviposition assessment was conducted at 1:00 pm with the presence of conspecific and heterospecific tracks. The arenas were kept in rectangular trays at $25 \pm 1^\circ\text{C}$, with a 14-hour photoperiod and RH of $75 \pm 5\%$. The data were subjected to the Kruskal-Wallis test followed by the Dunn test, using BioEstat 5.3 software at a significance level of 5%.

Evaluation of injuries

Tests. Three apple trees were used for injury assessments caused by *P. ulmi*, three for *T. urticae*, and three for *T. ludeni*, totaling three replicates per species for each treatment. Five leaves were isolated at the base of the petiole with entomological glue, each corresponding to a treatment. Each tree received a specific quantity of mites according to the corresponding treatment: Control: 0/leaf; T1: 10 mites/leaf (T10); T2: 25 mites/leaf (T25); T3: 50 mites/leaf (T50); T4: 100 mites/leaf (T100). The mites remained on the leaves for seven days, and the apple trees were kept at $25 \pm 1^\circ\text{C}$, with a 14-hour photoperiod and RH of $75 \pm 5\%$ during the experiment. After the period, the leaves were removed from the trees and sent for bioassays.

Chlorophyll index A, B, and total. To assess the total chlorophyll index, contaminated leaves were removed from the plants, and the assessment was immediately performed using an electronic chlorophyll content meter, ClorofiLOG CFL1030, Falker model. Ten random points were measured on each leaf. The measurement is made optically, based on the specific wavelengths of each chlorophyll. The means were subjected to the Kruskal-Wallis test followed by the Dunn test, using BioEstat 5.3 software at a significance level of 5%.

Membrane stability loss. For the evaluations of damage caused by *T. ludeni*, *T. urticae*, and *P. ulmi* on apple tree leaves, the histochemical analysis of plasma membrane stability loss, indicative of cell death, was performed using the method adapted from Romero-Puertas et al. (2004). For the analysis, a solution of 0.25% Evans Blue dye (0.25 g in 100 mL Milli-Q® Ultrapure water) was prepared. Subsequently, the leaves of each treatment were immersed in a Petri dish with the solution and remained at room temperature for five hours. Then, the leaves were removed from the solution and boiled in absolute alcohol, with a drop of glycerin for about an hour to remove the chlorophyll. During boiling, the Evans Blue dye penetrated the dead cells, marking the tissue with points or regions of bluish color due to damage caused by phytophagous mites. After boiling, the leaves were removed and preserved in a container with 70% alcohol for later photographic documentation.

Results

Oviposition of *Panonychus ulmi*. In terms of the effects of conspecifics and heterospecifics, 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. 1). The effect of the heterospecific *T. ludeni* on oviposition was similar to the control, while in the presence of webs and eggs of *A. schlechtendali* and *T. urticae*, there was a decrease in oviposition.

Regarding the effect of conspecific and heterospecific olfactory cues on the oviposition of *P. ulmi*, the previous cues of *P. ulmi* caused a significant increase in oviposition, differing from the others (Fig. 2). The presence of olfactory 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 the oviposition of *P. ulmi*.

Injury assessment. Leaves with *P. ulmi* showed significantly higher chlorophyll A indices in the control (C) and T10 compared to T25, T50, and T100 (Table 1). Regarding the content of chlorophyll B and total chlorophyll, the highest indices were found in C and T10, decreasing in T25 but with the lowest indices for T50 and T100. Leaves with *T. ludeni* followed the same response observed for *P. ulmi* regarding chlorophyll A. The indices of chlorophyll A, B, and total were significantly higher in C and T10 compared to T25, T50, and T100. Leaves with *T. urticae* had higher chlorophyll A, B, and total indices in the control compared to the other treatments. The lowest chlorophyll indices were observed for T50 and T100. When comparing chlorophyll levels between species, *T. urticae* showed a greater loss of chlorophyll in apple leaves. *Panonychus ulmi* and *T. ludeni* had higher levels of chlorophyll A, B, and total when compared to *T. urticae* in both C, T10, T25, and T50. In T100, *T. ludeni* showed a higher content of chlorophyll A, B, and total, while *P. ulmi* and *T. urticae* did not differ statistically.

Table 1

Evaluation of chlorophyll A, B, and total content (Mean $\pm$ SE) in apple leaves under different infestation levels of *Panonychus ulmi*, *Tetranychus ludeni*, *Tetranychus urticae* and Control (C), with 10 mites (T10), 25 mites (T25), 50 mites (T50), and 100 mites (T100).

Treatment	Chlorophyll								
	A			B			Total		
	<i>Panonychus ulmi</i>	<i>Tetranychus ludeni</i>	<i>Tetranychus urticae</i>	<i>P. ulmi</i>	<i>T. ludeni</i>	<i>T. urticae</i>	<i>P. ulmi</i>	<i>T. ludeni</i>	<i>T. urticae</i>
C	42.7 $\pm$ 0.32 Aa	43.2 $\pm$ 0.30 Aa	39.8 $\pm$ 0.33 Ba	18.1 $\pm$ 0.60 Aa	18.7 $\pm$ 0.49 Aa	14.3 $\pm$ 0.32 Ba	60.8 $\pm$ 0.88 Aa	61.9 $\pm$ 0.76 Aa	54.1 $\pm$ 0.59 Ba
T10	41.1 $\pm$ 0.33 Aa	41.9 $\pm$ 0.58 Aa	34.7 $\pm$ 0.33 Bb	16.6 $\pm$ 0.65 Aa	18.1 $\pm$ 0.55 Aa	8.5 $\pm$ 0.30 Bb	57.7 $\pm$ 0.93 Aa	60.1 $\pm$ 0.99 Aa	43.1 $\pm$ 0.51 Bb
T25	38.4 $\pm$ 0.45 Ab	39.2 $\pm$ 0.52 Ab	34.1 $\pm$ 0.44 Bb	13.2 $\pm$ 0.42 Ab	13.6 $\pm$ 0.70 Ab	8.2 $\pm$ 0.23 Bb	51.6 $\pm$ 0.89 Ab	52.8 $\pm$ 1.20 Ab	42.3 $\pm$ 0.62 Bb
T50	37.6 $\pm$ 0.30 Ab	39.2 $\pm$ 0.55 Ab	29.0 $\pm$ 0.70 Bc	11.7 $\pm$ 0.27 Abc	13.1 $\pm$ 0.53 Ab	6.0 $\pm$ 0.25 Bc	49.3 $\pm$ 0.52 Abc	52.3 $\pm$ 1.03 Ab	35.0 $\pm$ 0.87 Bc
T100	31.8 $\pm$ 1.47 Bb	36.0 $\pm$ 1.24 Ab	25.4 $\pm$ 1.47 Bc	8.1 $\pm$ 0.65 Bc	12.8 $\pm$ 1.04 Ab	6.6 $\pm$ 0.40 Bc	39.9 $\pm$ 2.14 Bc	48.8 $\pm$ 2.18 Ab	32.0 $\pm$ 1.81 Bc
*Means (Mean $\pm$ SE) followed by the same lowercase letter within a column in the treatments do not differ statistically from each other by the Kruskal-Wallis test, followed by Dunn's test ($\alpha = 0.05$).									
**Means (Mean $\pm$ SE) followed by the same uppercase letter in the row, the species do not differ statistically in terms of chlorophyll by the Kruskal-Wallis test followed by Dunn's test ($\alpha = 0.05$).									

In the histochemical analysis of plasma membrane stability loss (indicative of cell death), similar levels were observed in leaves infested by *P. ulmi* in C, T10, T25, and T50, with a higher intensity of injuries in leaves with T100 (Fig. 3). In leaves infested with *T. ludeni*, C, T10, and T25 showed similar levels of membrane stability loss, while in T50 and T100, there was a higher intensity of loss. In leaves infested with *T. urticae*, injuries were less visible, with no blue coloration representing mite attacks in both C and T10, T25, and T50; some points were observed in T100.

Discussion

The results of this study suggest that the presence of conspecific eggs, webs, and olfactory cues increases the oviposition of *P. ulmi* on apple leaves, indicating that the species performs better in environments without heterospecific competitors and where conspecifics have been and demonstrated safety for the development of future generations. However, there is a decrease in oviposition of *P. ulmi* in the presence of *A. schlechtendali*, indicating a potential competitive displacement of the Tetranychid by the Eriophyid mites.

The previous presence of mites of the same species may have favored oviposition and the 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 risky situations (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 indicate less competition for resources (Mayhew 1997; Gripenberg et al. 2010). Thus, new populations of *P. ulmi* may have perceived the location as a favorable environment for the establishment of their offspring as a survival strategy for the species.

The presence of *T. urticae* (web and egg) causing a decrease in the oviposition of *P. ulmi* on apple leaves may indicate competition between the species (Ferragut et al. 2013; López-Olmos and Ferragut 2023). Moreover, the decrease in oviposition of *P. ulmi* in the presence of webs and olfactory cues of *A. schlechtendali* may indicate a competitive displacement of the Tetranychid due to the presence of the Eriophyid, as observed by Croft and Hoying (1977). There are reports of competitive displacement due to the coexistence of species that use and compete for the same spaces and resources (López-Olmos and 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 mite species (Reitz and Trumble 2002). Therefore, specific studies on the competition between phytoseiid mite species present in apple orchards are recommended.

The 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 and Sabelis 1988; Dicke et al. 1990). Similar to many studies demonstrating the influence of volatiles released by plants on predator-prey behavior in a tritrophic context, these kairomones can be compounds associated with the herbivores themselves present in their feces, eggs, exuviae, webs, and pheromones (Collier et al. 2000; Afsheen et al. 2008; Peñaflor 2019), mediating the behavior and interaction among organisms (Collier et al. 2000; Ayelo et al. 2021).

Regarding injuries, the infestation level of different phytophagous species influenced the chlorophyll index of the apple trees, with higher infestations of *T. urticae* causing a more significant decrease in chlorophyll levels. The loss of membrane stability was also more affected by higher infestation levels, but in this case, the species responsible for the higher cell death was *T. ludeni*. These different observed responses may be related to the distinct damages caused by each acarine species on the plant leaves and can help elucidate the damage threshold for each species in question.

Arthropods, in general, damage the physiological metabolism of plants, with piercing through stylets breaking cells, potentially reaching the photosynthetically active mesophyll tissue, leading to the destruction of chloroplasts (Bueno et al. 2009). Thus, reductions in photosynthetic pigments can be explained due to injuries caused by the mite, as it sucks cellular content, extracting cytoplasmic content from the cells (Nagara et al. 2002). The significant decrease in chlorophyll levels in apple plants caused by *T. urticae* is likely a result of the metabolically active secretions of the species, which may have interacted with the plant's photosynthetic mechanism, causing a greater decrease in chlorophyll indices (Nagara et al. 2002). Also, the aggressiveness of the species may contribute, as cells not directly damaged but adjacent to those that suffered harm may exhibit mild symptoms (Park and Lee, 2002).

The evaluations further showed that the level of phytophagous infestation on leaves influences the chlorophyll content, with infestations from 25 mites causing a decrease in chlorophyll indices. The higher the infestation, the lower the chlorophyll content. Thus, as the number of mites increased, more cells were damaged, leading to a greater reduction in chlorophyll content and the plant's photosynthetic rate. The photosynthetic rate is the plant parameter

most sensitive to the relationship between yield and pest abundance (Xu and Zhang 1991). Some plants may recover to some extent after short periods of infestation, but longer feeding periods significantly reduce the plants' recovery capacity (Nagaraj et al. 2002). In this study, high mite populations continued feeding on apple plants for seven days, which may have caused irreversible damage to the plants' photosynthetic mechanism, with significant reductions in chlorophyll levels. Additionally, it is necessary to consider that there may be indirect damage to the photosynthetic mechanism that may not necessarily manifest as chlorosis (Nabity et al. 2009).

When evaluating the leaves for membrane stability loss, the dark blue staining detected through histochemical staining using Evans Blue reagent indicates the detection of higher levels of injuries caused by phytophagous mites to apple leaves. Thus, greater loss of membrane stability was also observed in treatments with higher mite infestation for the three evaluated species. In addition to direct cellular damage, as evidenced by the loss of photosynthetic capacity, mite-induced injury to the plant leaves can lead to oxidative damage, inducing lipid peroxidation, and with redox imbalance causing membrane instability (Santamaría et al. 2018). Besides the partial destruction of cells due to the withdrawal of their cellular content, there are also various lesions from stylet piercing, and the disintegration and decomposition of organelles, likely due to the action of proteolytic enzymes secreted by mite saliva (Kielkiewicz 1985).

The evaluations demonstrate that infestations above 50 mites cause membrane stability loss, leading to cell death promoted by high mite infestation levels, similar to the findings of Blasi et al. (2017). Injuries were observed within seven days of the onset of infestation, particularly in infestations exceeding 25 mites per leaf. Moreover, visible injuries were observed in leaves that received infestations from 50 mites within the same period. Blasi et al. (2017), in studies conducted with rice leaves (*Oryza sativa* L.) infested with high population levels of *Schizotetranychus oryzae* Rossi de Simons (Tetranychidae) after 30 days, caused a significant reduction in grain productivity. Furthermore, in this study, the most pronounced signs of cell death in apple leaves were caused by *T. ludeni*, demonstrating its potential to become an economically important species in cultivation.

Studies like this are essential as they enable the understanding of the effects of different species of phytophagous mites on apple trees and their interaction with the environment and other species, assisting in decision-making regarding the most appropriate management practices for cultivation. Additionally, this work provides unprecedented information about injuries caused by *T. ludeni* in apple trees, as it is an uncommon species in this context (Rode et al. 2023). The presented results underscore the potential of this species as a possible cause of economic damage if it reaches apple orchards.

Conclusions

In conclusion, it can be inferred from this study that the previous presence of conspecifics increases the oviposition of *P. ulmi* on apple leaves, while the presence of heterospecifics (*A. schlechtendali*) decreases *P. ulmi* oviposition. Furthermore, the higher the number of mites on the leaf surface, the greater the intensity of damage and the larger the damaged area. *T. urticae* is responsible for the most significant decrease in the chlorophyll index of apple leaves, while *T. ludeni* has the highest potential for causing cell death in apple leaves. Therefore, this study serves as a warning to the apple production chain to monitor potential infestations at the field level, and it recommends that more in-depth studies be conducted to prevent a possible threat to apple cultivation. These results are promising as they provide a foundation for further research on the potential of phytophagous mite species in apple trees, especially *T. ludeni* and *A. schlechtendali* (recently recorded in cultivation), and research related to the interaction between these herbivores and their predators, as well as with apple trees.

Declarations

Competing Interests

The authors have no relevant financial or non-financial interests to disclose.

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Author Contribution

P.A.R. with the methodology, analysis, interpretation of results and concept of the manuscript; J.R.S. with interpretation of results and revised final version; C.J.C.M. with the analysis; R.D.M. and I.S.C. with the methodology (maintenance of mites); N.J.F. with revisions.

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Figures

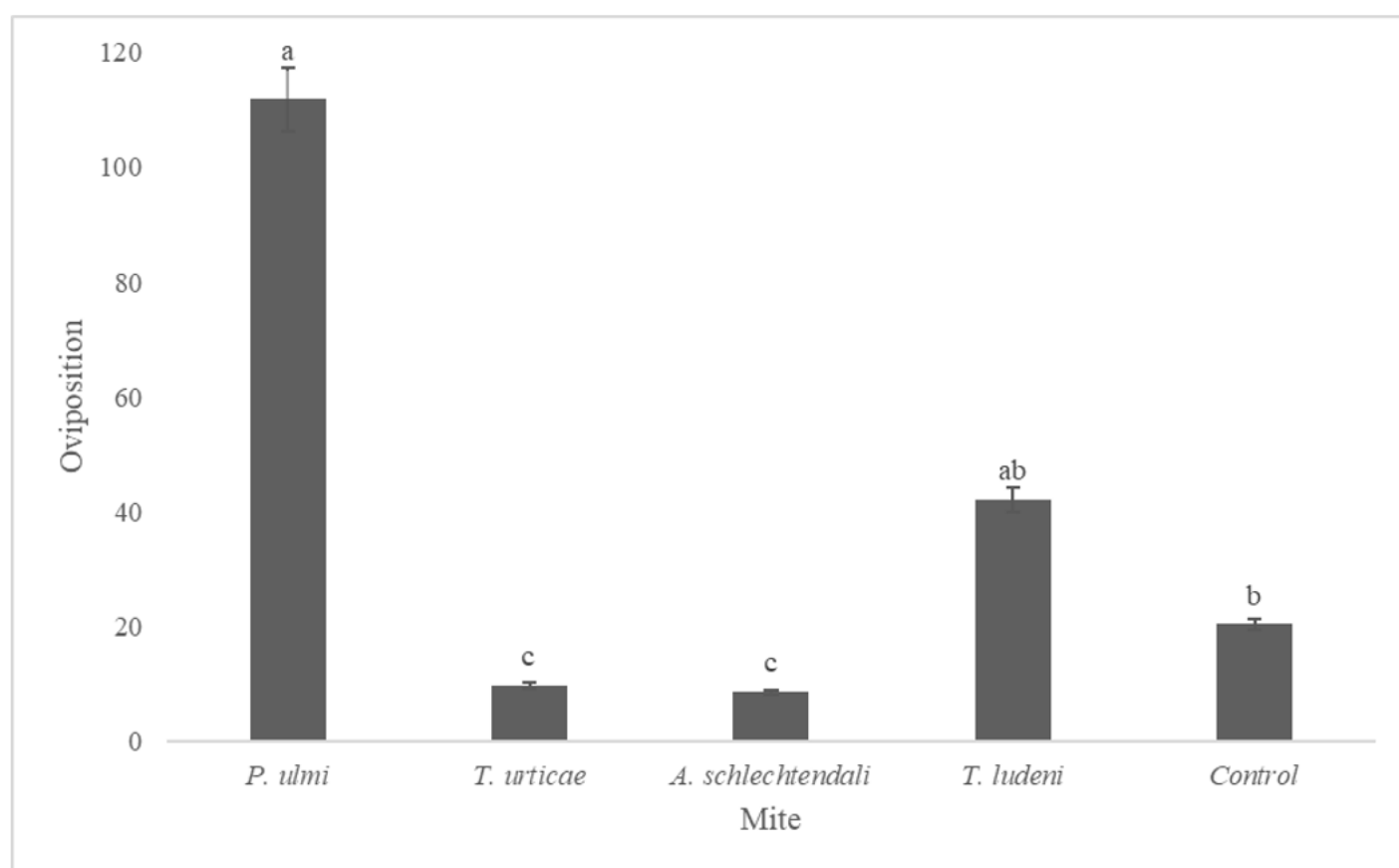


Figure 1

Mean oviposition rate of *Panonychus ulmi* on apple leaf disks with injury, webbing, and eggs of *Aculus schlechtendali*, *Panonychus ulmi*, *Tetranychus ludeni*, *Tetranychus urticae*, and control, over a seven-day period.

Different letters denote significant differences between treatments by Kruskal-Wallis test, followed by Dunn's test ($\alpha = 0.05$).

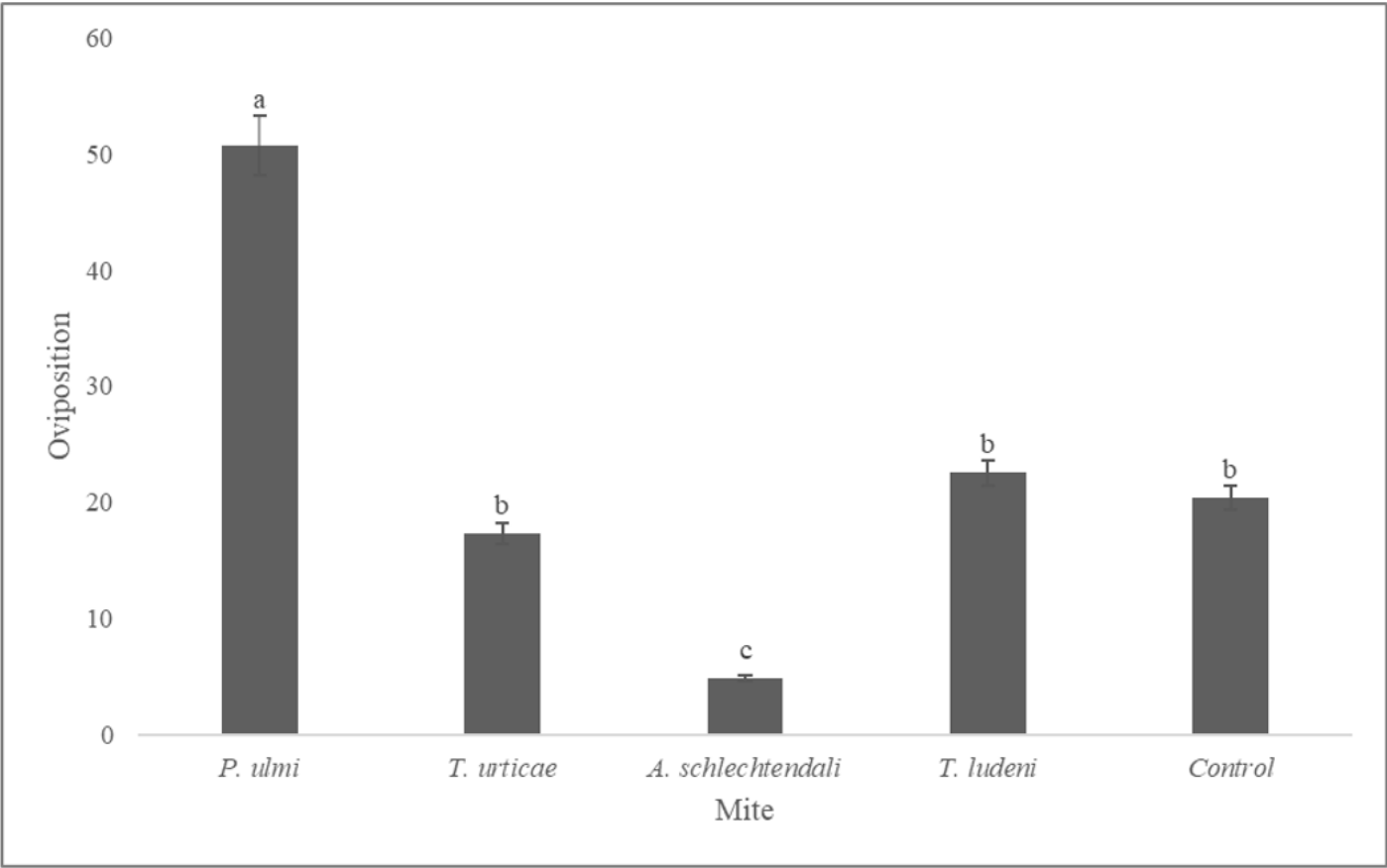


Figure 2

Mean oviposition rate of *Panonychus ulmi* on apple leaf disks with injury and cues of *Aculus schlechtendali*, *Panonychus ulmi*, *Tetranychus ludeni*, *Tetranychus urticae*, and control, over a seven-day period. Different letters denote significant differences between treatments by Kruskal-Wallis test, followed by Dunn's test ($\alpha = 0.05$).

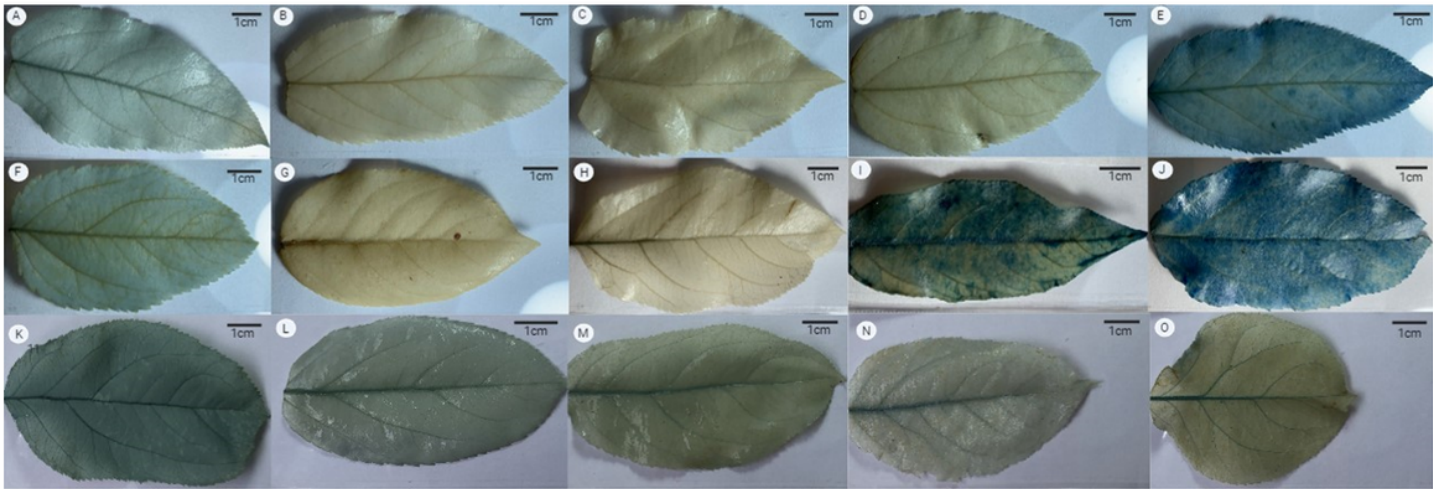


Figure 3

Injuries caused to apple leaves by *Panonychus ulmi* - A- Control, B- 10 mites, C- 25 mites, D- 50 mites, E- 100 mites; *Tetranychus ludeni* - F- Control, G- 10 mites, H- 25 mites, I- 50 mites, J- 100 mites; *Tetranychus urticae* - K- Control, L- 10 mites, M- 25 mites, N- 50 mites, O- 100 mites.