

# *Galendromus occidentalis* (Acari: Phytoseiidae) life table parameters on *Oligonychus yothersi* (Acari: Tetranychidae) colonies and its behavior to odors of mites, avocado shoots volatiles and synthetic compounds

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Received: 15 July 2021; Accepted: 17 November 2021; doi:10.4067/S0718-58392022000100124

## ABSTRACT

The red mite, *Oligonychus yothersi* (McGregor) (Acari: Tetranychidae), is a serious pest of avocado (*Persea americana* Mill.) ‘Hass’ in Chile. Micro-coleopterans predators are observed in avocado orchards during outbreaks of *O. yothersi*, which are attracted by herbivore-induced plant volatiles (HIPVs). However, the damage to plants persists and new predators are needed. Due to its effectiveness and adaptability to dry climates, the western predatory mite *Galendromus occidentalis* (Nesbitt) (Acari: Phytoseiidae) has been adopted as a biological control agent on many crops. Our objectives were to study the biological parameters of *G. occidentalis* preying on *O. yothersi* in controlled conditions and its attraction to volatile compounds from avocado shoots, live preys and four synthetic doses of methyl salicylate (MeSA) and ocimene using a Y-tube olfactometer. We found a net reproductive rate ( $R_0$ ) 34.41 offspring female<sup>-1</sup>, intrinsic rate of increase ( $r_m$ ) 0.19 females female<sup>-1</sup> d<sup>-1</sup>, finite rate of increase ( $\lambda$ ) 1.21 females female<sup>-1</sup>, mean generation time (T) 18.45 d, and doubling time (DT) of 3.61 d. Immature phytoseiids took 7.32 d to reach adulthood with 90% survival. Female longevity and fecundity were 36.27 d, and 59 eggs female<sup>-1</sup>, respectively. Phytoseiids show no attraction to *O. yothersi* volatiles or volatiles of *O. yothersi*-infested avocado shoots. Nevertheless, females showed a preference for synthetic MeSA and ocimene at 100  $\mu\text{g mL}^{-1}$ . Our findings indicate that *O. yothersi* is a potential diet to rear *G. occidentalis*, and MeSA and ocimene could be used in lures to manipulate its behavior in avocado orchards.

**Key words:** Avocado shoot volatiles, behavioral response, life table parameters, red mite, western predatory mite.

## INTRODUCTION

Phytophagous mites belonging to the Tetranychidae family are serious pests worldwide, attacking crops, tree fruits and ornamental species. These mites use their stylet-like chelicerae to feed, piercing-sucking cell contents, provoking mechanical damage, discolorations, and reddish color by oxidative stress in leaves tissue. As a result, emissions of herbivore-induced plant volatiles (HIPVs) (Rioja et al., 2016; Erb and Reymond, 2019), such as terpenoids, green leaf volatiles (GLVs), jasmonates and esters as methyl salicylate (MeSA) (Aartsma et al., 2017) are elicited.

Over the past decades, the production of avocado (*Persea americana* Mill.) has increased at a rate of 3.7% annually, registering 6470171 t in 2018, cultivated in 918531 ha (FAOSTAT, 2021). Chile is one of the main ‘Hass’ avocado exporters worldwide, exporting roughly 168000 t during 2019/2020 season (ASOEX, 2020), harvested from 29.186 ha, being 55%-65% of this area concentrated in the Valparaíso Region (ODEPA, 2018).

In Chile, *Oligonychus yothersi* (McGregor) (Acari: Tetranychidae) is the most severe foliar pest of avocado 'Hass' orchards. The *O. yothersi* outbreaks occur between mid-summer and late autumn at Valparaíso Region (Rioja et al., 2019). Beetles *Oligota pygmaea* Solier (Coleoptera: Staphylinidae) and *Parastethorus histrio* Chazeau (Coleoptera: Coccinellidae) are observed preying all stages of *O. yothersi* on damaged and infested avocado trees (Hoy, 2011; Rioja et al., 2016). Since both coleopterans are dense-dependent, they require higher mite populations to establish themselves, and HIPVs released by *O. yothersi*-infested avocado recruit these predators (Rioja et al., 2018). The control of the red mite is achieved by pesticides usage, mineral oils, and aerial sulfur applications (Ripa and Larral, 2008). However, some studies have reported susceptibility and mortality of *Stethorus punctillum*, *S. punctum picipes*, and *S. nigripes* (Coleoptera: Coccinellidae) to pesticides and sulfur (Hoy, 2011; Gesraha and Ebeid, 2019), making challenging their incorporation into integrated pest management (IPM) programs. Hence, new predators adapted to different prey availability and pesticides tolerance are required to avoid foliar damage in avocado plants.

The western predatory mite *Galendromus* (= *Typhlodromus*, = *Metaseiulus*) *occidentalis* (Nesbitt) (Acari: Phytoseiidae) is a type II lifestyle phytoseiid, associated with dense web producing mites, which are selective to a wide range of tetranychid mites, including *Oligonychus* and *Tetranychus* species (McMurtry et al., 2013). They are used in mite management programs on apples (*Malus domestica* (Suckow) Borkh.), almonds (*Prunus dulcis* (Mill.) D.A. Webb), walnuts (*Juglans regia* L.), strawberries (*Fragaria xananassa* Duchesne ex Rozier) and greenhouses for its adaptability to dry climates, pesticide resistance, and persistence at low prey density (Hoy, 2011; Schmidt et al., 2013). In addition, *G. occidentalis* have been reported as an effective predator on *Oligonychus perseae* (Acari: Tetranychidae) in avocado plants (McMurtry et al., 2013).

The biological parameters of predators are crucial to evaluate the effectiveness against mite pests and to predict the potential suppression of mite populations. Several studies have explored the postembryonic development of *G. occidentalis* fed with *Tetranychus urticae* Koch and *T. pacificus* McGregor (Acari: Tetranychidae) (Laing, 1969; Badii and McMurtry, 1984). Furthermore, life table reports on *G. occidentalis*, being fed with eggs of *T. pacificus*, have signaled high net reproductive rate ( $R_0$ ) and intrinsic rate of increase ( $r_m$ ), unlike females fed with a mixed of larvae, protonymphs, and deutonymphs (Bruce-Oliver and Hoy, 1990). There has also been previous evidence of life table parameters of *G. occidentalis* supplying a strict daily diet of *O. yothersi* (10 immatures) (Rioja and Vargas, 2009). Along with that, Yanar and Hoy (2015) studied biological parameters of *G. occidentalis* after different intervals at low temperatures to determine the storage potential for its commercialization and use in biological control programs; their results reveal that *G. occidentalis* can be stored at 7 °C or 10 °C for up to 4 wk. Therefore, life table parameters of *G. occidentalis* on *O. yothersi* populations are of substantial importance to examine the maximum of its potential as a biological control agent.

Predatory mites are attracted by chemical cues from mite-infested plants, where species as *Phytoseiulus persimilis* Athias-Henriot, *Neoseiulus baraki* (Athias-Henriot) and *P. macropilis* (Banks) (Acari: Phytoseiidae) have shown attraction to blends of HIPVs from candy mint (*Mentha x piperita* L. 'Candy'), strawberry (*Fragaria xananassa*), and coconut (*Cocos nucifera* L.) (Fadini et al., 2010; Melo et al., 2011; Togashi et al., 2019). Predators associate HIPVs with prey availability for their offspring, and detect kairomones from feces and webbing from mites using its sensory receptors seta-like on legs I and on palps (Hoy, 2011). In addition, some studies have evaluated the attraction of predatory mites toward synthetic HIPVs as terpenoids, GLVs, and MeSA (Shimoda, 2010; Gadino et al., 2012b).

We hypothesize that *G. occidentalis* prey on *O. yothersi*, developing and completing its life cycle, and use chemical cues as HIPVs to find prey and host plants. We studied the biological parameters of *G. occidentalis* on avocado leaf discs infested with *O. yothersi* and its olfactory responses toward *O. yothersi* volatiles, avocado shoots HIPVs, and synthetic doses of MeSA and ocimene in laboratory conditions, to determine its potentiality as biological control agent.

## MATERIALS AND METHODS

### Avocado red mite and phytoseiids rearing procedures

We reared *Oligonychus yothersi* (McGregor) (Acari: Tetranychidae) on avocado (*Persea americana* Mill.) 'Hass' leaf discs (diameter = 8.5 cm) on wet cotton in plastic Petri dishes at  $23 \pm 3$  °C,  $50 \pm 10\%$  RH and 16:8 h photoperiod (Rioja et al., 2016). The phytoseiid females were extracted from colonies maintained in black acrylic dishes (diameter = 12 cm), according to the method described by Swirski et al. (1970) and fed with *T. urticae* (Acari: Tetranychidae). Large

deutonymphs (which originate females individuals) and males of *G. occidentalis* were isolated in a new acrylic dish using a 40X stereoscopic magnifying glass (Zeiss, Stemi, Göttingen, Germany) to obtain 48-h-old gravid females to behavioral responses in two-choice tests. The rearing conditions were  $25 \pm 3$  °C,  $60 \pm 10\%$  RH and 16:8 h photoperiod.

### Postembryonic development bioassays

The *Galendromus occidentalis* (Nesbitt) (Acari: Phytoseiidae) females were reared on *O. yothersi*-infested avocado leaf discs (diameter = 8.5 cm) into a plastic Petri dish; to avoid their escape, we applied Stickem (Seabright Laboratories, Emeryville, California, USA) to leaf discs. After 4 h we removed females, allowing one egg per leaf disc (N = 30). Every 24 h, we registered the egg-adult development, longevity and survival, and we used exuviae to differentiate developmental stages, which was later discarded with a fine brush. We incubated the Petri dishes at  $25 \pm 0.7$  °C,  $60 \pm 5\%$  HR, and 16:8 h photoperiod (Thermo Scientific Precision Model 818 Incubator, Marietta, Ohio, USA), and performed all observations using a 40X stereoscopic magnifying glass (Zeiss, Stemi, Göttingen, Germany).

### Life table parameters

A newly emerged *G. occidentalis* female was carefully transferred on an avocado infested-leaf disc into a plastic Petri dish, with two males for 24 h every 7 d, to ensure copulation. We recorded the number of eggs laid on each leaf disc, which was eliminated every 24 h; we considered a female as a replicate (N = 15). To get the sex ratio and number of *G. occidentalis* female offspring, every 24 h we randomly selected 10 replicates and counted the laid eggs. Then, we transferred the eggs of *G. occidentalis* to new infested-leaf discs. Sex ratios of the *G. occidentalis* offspring were determined by body size and opisthosoma shape (sexual dimorphism) using a 40X stereoscopic magnifying glass (Zeiss, Stemi, Göttingen, Germany). We incubated the Petri dishes in at  $25 \pm 0.7$  °C,  $60 \pm 5\%$  HR, and 16:8 h photoperiod.

### Avocado infestation and volatile collection

Twelve 2-yr-old avocado 'Hass' plants grafted on 'Mexicola' rootstock were used in volatile collections. We selected a shoot (15-30 cm tall) per plant, with 4 to 6 fully developed leaves to infest it with  $250 \pm 10$  mites per leaf by attaching a leaf disc containing *O. yothersi*. All infested leaf discs were obtained from *O. yothersi* rearing described above (Rioja et al., 2016). Six avocado 'Hass' plants were infested and kept into a white muslin greenhouse (3 × 2.5 × 4 m) under semi-field conditions in La Cruz (32°49' S; 71°17' W), Valparaíso Region, Chile. The red mites could feed freely until volatile collection; to avoid their escape, we applied Stickem (Seabright Laboratories, Emeryville, California, USA) to the base of the selected shoots. Subsequently, the number of mites of each leaf was corroborated using a 10× handheld magnifying glass. The uninfested control plants (N = 6) were isolated in a separate white muslin greenhouse. All volatile collections were performed 3 d after infestation procedure.

As described in Rioja et al. (2018) we used dynamic headspace technique with a positive/negative pressure air system. We performed collections for 24 h enclosing shoots into 1.5 L polyethylene terephthalate (PET) bottles cut vertically and sealed with Teflon tape. Volatiles were gathered onto 100 mg Porapak Q traps (80-100 mesh, Waters Associates, Milford, Massachusetts, USA), previously cleaned and conditioned at 150 °C for 2 h in a stream of 70 mL N min<sup>-1</sup> (Ceballos et al., 2015). We eluted the volatiles with 1 mL hexane (Chromatographic grade, Optima Scientific, Green Bay, Wisconsin, USA) and stored them at -20 °C until their analysis.

### Chemical analysis of avocado volatiles

One microliter of volatile extracts was injected into a gas chromatographer coupled to a mass spectrometer (GC-MS) (QP2010 Ultra, Shimadzu, Kyoto, Japan), equipped with an RTx5 capillary column (30 m, 0.25 mm ID, 0.25 µm film thickness; Restek, Bellefonte, Pennsylvania, USA). The injection mode was splitless, and helium was used as carrier gas at 1.0 mL min<sup>-1</sup>. We set oven temperature at 40 °C and held for 2 min; then, it was increased at a rate of 5 °C min<sup>-1</sup> until reaching 225 °C. We programmed the interface temperature at 250 °C and performed the mass spectrum acquisition in the range from 35 to 500 m/z. Electron impact performed the ionization at 70 eV with an ion source at 200 °C, and volatiles compounds were identified by comparing their retention times and mass spectrums with the NIST05 database (National Institute of Standards and Technology [NIST], Gaithersburg, Maryland, USA) library and commercial standards, and quantified using the internal standard method.

### Behavioral responses to volatile compounds

We studied the olfactory responses of *G. occidentalis* to volatile compounds by two-choice tests employing a glass Y-tube as a behavioral arena (8 cm arm length, 1 cm internal diameter, and 60° angle). A Y-shaped iron wire was positioned in the center of Y-tube as a path for the movement of phytoseiids. As odor sources, we used volatiles emitted by 200 lives of *O. yothersi* females. We also used avocado shoots volatiles, collected as described above, and four doses in hexane (1, 10, 100, and 1000 µg mL<sup>-1</sup>) of commercial synthetic standards of methyl salicylate (MeSA) and ocimene. We designated the two opposite arms as control and stimulus zones, and the base of the Y-tube as the decision zone. One *G. occidentalis* female was released at a time in the decision zone, and we recorded the time spent to visit each zone of the arena for 10 min. We fed *G. occidentalis* with *T. urticae* without a host plant to avoid the learning phenomenon reported in phytoseiidae (Christiansen et al., 2016). We loaded 50 µL eluted volatiles from either infested or uninfested avocado shoots or the four doses of commercial standards onto a paper strip (7 cm × 5 mm, Whatman nr 1 filter paper), and placed it into a glass tube (100 mm long and 10 mm diameter) attached to each arm of the olfactometer and pulled at 300 mL min<sup>-1</sup> by a vacuum pump (BOECO, Hamburg, Germany) connected to the base of the Y-tube. The volatiles from the avocado red mite were collected enclosing 200 lives females of *O. yothersi* for 2 h into a glass tube (100 mm long and 10 mm diameter) and connected to the stimulus arm, while clean air passed through the control arm. We performed 20 replicates, and we tested each individual once, using a different and clean olfactometer. The olfactometer was rotated 90° (clockwise) after each replicate to minimize directional bias, and we carried all bioassays out between 10:00 and 15:00 h, under laboratory conditions (25 ± 2 °C and 65 ± 5% RH).

### Statistical analysis

We developed the *G. occidentalis* female age-specific life table calculating the parameters: net reproductive rate ( $R_0 = \sum l_x m_x$ , number of females that produce a female during a generation or during their lifespan); intrinsic rate of increase ( $r_m$ ) being the maximum exponential multiplication rate of an entire population, and we calculated it using  $\sum e^{-r(x+1)} (l_x m_x) = 1$ , females female<sup>-1</sup> d<sup>-1</sup>; finite rate of increase ( $\lambda = e^{r_m}$ ), multiplication factor of a population per female at each period time; mean generation time ( $T = (\ln R_0)/r_m$ ), being the time that passes between first and next generation; and doubling time ( $DT = (\ln 2)/r_m$ ) (Birch, 1948). We applied the jackknife method to obtain pseudo-values for each parameter, and their means were calculated (Meyer et al., 1986). For behavioral bioassays, we analyzed the spent time on the control or stimulus zones through the *U* Mann-Whitney test ( $P < 0.05$ ), and to compare the doses of synthetic compounds, we applied the nonparametric one-way procedure of Kruskal-Wallis followed by Dunn's multiple comparison test ( $P < 0.05$ ).

## RESULTS AND DISCUSSION

### Development and longevity of *Galendromus occidentalis* on *Oligonychus yothersi* colonies

The postembryonic development of *G. occidentalis* took 4.5 d, reaching adulthood 90% of them (Table 1), showing a high survival rate. In addition, the sex ratio was 60.8% females and 39.2% males on *O. yothersi* colonies. The biological studies of this phytoseiid species are limited. *Galendromus occidentalis* reared on bean leaves (4 cm<sup>2</sup>) and fed with *T. pacificus* registered 6.3 d from egg-to-adult (Badii and McMurtry, 1984); while immatures of *G. occidentalis* fed with *T. urticae* (20 eggs d<sup>-1</sup>) and maintained on strawberry leaflets lasted between 7.2 and 9.4 d (Laing, 1969). *Galendromus occidentalis* reared with *O. yothersi* (10 immatures d<sup>-1</sup>) took 8.6 d to complete its development (Rioja and Vargas, 2009). In our results, phytoseiids lasted 7.3 d until the adult stage. However, when they were offered strict daily diets, its development time increased at 8.6 or 9.4 d. Hence, *O. yothersi* colonies had a positive influence on its postembryonic development since availability of all prey stages and mite nests would affect the *G. occidentalis* development.

### Life table parameters of *Galendromus occidentalis* preying on *Oligonychus yothersi* colonies

Laing (1969) found that females of *G. occidentalis* fed with *T. urticae* lasted 3.2 d to lay eggs, while oviposition time was extended to 15.9 d. Badii and McMurtry (1984) fed *G. occidentalis* with *T. pacificus* eggs and registered an oviposition time of 19 d. Nevertheless, in our study, females of *G. occidentalis* reared on *O. yothersi* showed a lower pre-oviposition period (2.2 d), and long oviposition time (29.6 d) (Table 2). The survival rate ( $l_x$ ) of females reared on *O. yothersi* colonies was maintained at 90% for 23 d; then it was decreased to 88% for 8 d, and finally decreased promptly (Figure 1). The

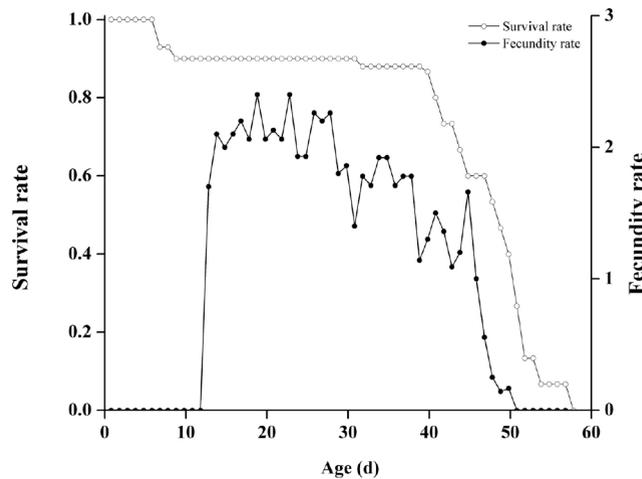
**Table 1. Post-embryonic development, longevity and survival of *Galendromus occidentalis* (mean  $\pm$  SE) feed on *Oligonychus yothersi*.**

Stage	Survival	Development
	%	d
Egg	100	3.16 $\pm$ 0.17
Larva	100	1.24 $\pm$ 0.10
Protonymph	93	1.57 $\pm$ 0.10
Deutonymph	90	1.68 $\pm$ 0.11
Egg-adult	90	7.32 $\pm$ 0.26
Female longevity		36.27 $\pm$ 1.71

**Table 2. Reproductive parameters of *Galendromus occidentalis* (mean  $\pm$  SE) on avocado leaf discs infested with *Oligonychus yothersi*.**

Reproductive parameter	
Pre-oviposition period, d	2.20 $\pm$ 0.11
Oviposition, d	29.66 $\pm$ 1.59
Post-oviposition, d	4.40 $\pm$ 0.38
Fecundity, egg female <sup>-1</sup> d <sup>-1</sup>	1.83 $\pm$ 0.04

**Figure 1. Age-specific survival rate ( $l_x$ ) and age-specific fecundity rate ( $m_x$ ) of *Galendromus occidentalis* females on avocado leaf discs infested with *Oligonychus yothersi*.**



fecundity of *G. occidentalis*, registered by us, was similar to the fecundity of females fed with *T. pacificus* eggs (1.84 eggs female<sup>-1</sup> d<sup>-1</sup>) (Badii and McMurtry, 1984). Bruce-Oliver and Hoy (1990) described that *G. occidentalis* fed with *T. pacificus* (mixed actives) would increase its population 22.02 times ( $R_0 = 22.02$ ) during the mean generation time (17 d); yet, when they were fed with *T. pacificus* eggs, its population increased 31.11 times in 14.1 d.

Rioja and Vargas (2009), on the other hand, registered a lower net reproductive rate ( $R_0 = 16.25$ ) of *G. occidentalis* fed with 15 immatures of *O. yothersi* d<sup>-1</sup>. In this study we registered a higher  $R_0 = 34.4$  females female<sup>-1</sup> when *G. occidentalis* were reared on *O. yothersi* colonies (Table 3), suggesting that the availability of all prey stages, nest structures and mite residues would increase their  $R_0$ , since it is a type II lifestyle phytoseiid, associated to tetranychid colonies with dense

**Table 3. Life-table parameters of *Galendromus occidentalis* (mean  $\pm$  SE) on avocado leaf discs infested with *Oligonychus yothersi*.**

Parameter	
Net reproductive rate ( $R_0$ ), offspring female <sup>-1</sup>	34.406 $\pm$ 1.739
Intrinsic rate of increase ( $r_m$ ), females female <sup>-1</sup> d <sup>-1</sup>	0.192 $\pm$ 0.002
Finite rate of increase ( $\lambda$ ), females female <sup>-1</sup>	1.211 $\pm$ 0.002
Mean generation time (T), d	18.452 $\pm$ 0.260
Time to doubling its population (DT), d	3.613 $\pm$ 0.028

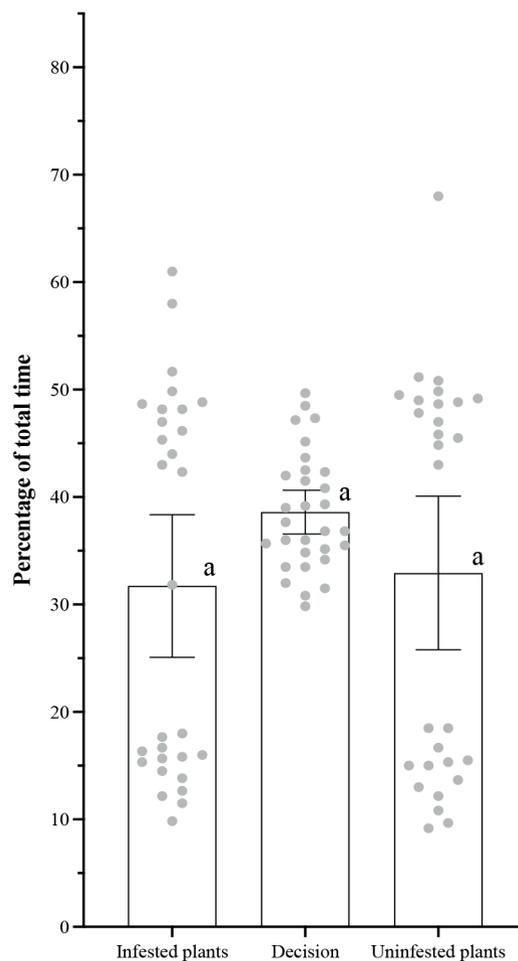
webbing (McMurtry et al., 2013). Thus, each female of *G. occidentalis* can produce 34.4 females' offspring and double its population in 3.6 d on *O. yothersi* colonies, showing that this prey would be suitable for rearing at laboratory conditions and for field studies.

The intrinsic rate of increase ( $r_m$ ) is an important index to evaluate predators as biological control agents, where predators'  $r_m$ -values must be higher than those values of pests. *Galendromus occidentalis* fed with mobile stages of *T. pacificus* got a  $r_m = 0.20$  females female<sup>-1</sup> d<sup>-1</sup> (Bruce-Oliver and Hoy, 1990). Ganjisaffar and Perring (2017) recorded a similar  $r_m$  value for *Galendromus flumenis* (Acari: Phytoseiidae) reared with eggs of *Oligonychus pratensis* (Banks) (Acari: Tetranychidae). Our results yielded a  $r_m = 0.19$  females female<sup>-1</sup> d<sup>-1</sup>, when they preyed on all stages of *O. yothersi*. This  $r_m$  value is lower than  $r_m = 0.24$  of red mite *O. yothersi* (Rioja et al., 2019). However, *G. occidentalis* is an efficient consumer of eggs and active stages of *T. pacificus* (Bruce-Oliver and Hoy, 1990; Hoy, 2011), being effective in the control of *Oligonychus perseae* Turtle, Baker and Abbatiello (Acari: Tetranychidae) (McMurtry et al., 2013).

### Behavioral responses of *Galendromus occidentalis*

In two-choice tests *G. occidentalis* females did not discriminate between odors from uninfested and infested avocado shoots (Figure 2). Nonetheless, Melo et al. (2011) reported that *N. baraki* (Phytoseiid) was attracted to coconut (*C. nucifera*) infested with *Aceria guerreronis*, and Fadini et al. (2010) found that *P. macropilis* showed attraction to *T. urticae*-infested strawberry plants. Furthermore, Maleknia et al. (2013) studied the olfactory responses of *P. persimilis* using two-choice bioassays; found that *P. persimilis* were significantly attracted to *T. urticae*-infested bean (*P. vulgaris*

**Figure 2. Olfactory responses of *Galendromus occidentalis* to volatiles emitted by avocado plants in Y-tube olfactometer.**



Mean spent time ( $\pm$  SD). Bars sharing a letter do not significantly differ according to the U Mann-Whitney test ( $P < 0.05$ ). A grey dot represents the spent time by a tested individual ( $N = 30$ ).

‘Alamot’). Likewise, the phytoseiids preferred odors from *T. urticae*-infested cucumber (*Cucumis sativus* L. hybrids ‘Negin’) plants. *Phytoseiulus persimilis*, on the other hand, did not show preference to odors from *T. urticae*-infested rose or uninfested rose (*Rosa* L. hybrids ‘Black Magic’) plants. It is known that the predators are attracted to HIPVs, triggering tri-trophic interactions as co-evolutionary processes and adaptations (Kergunteuil et al., 2020). Our results reveal that *G. occidentalis* does not associate the HIPVs released by *O. yothersi*-infested avocado shoots (Table 4) with prey availability since they do not belong to the avocado system. In contrast, *P. histrio* and *O. pygmaea* are attracted to HIPVs emitted by *O. yothersi*-infested avocado shoots (Rioja et al., 2018). These predators use chemical cues from avocado-*O. yothersi* system, which shows food availability and shelter for its offspring.

Phytoseiids detect chemical cues released by phytophagous mites and those emitted by its feces, webbing, molt residues serving as kairomones (Hoy, 2011). Gencer et al. (2009) showed that *T. urticae* kairomones were significantly attractant to *Stethorus gilvifrons* (Mulsant) (Coleoptera: Coccinellidae). In our study, we offered *O. yothersi* volatiles vs. clean air in two-choice tests; our results indicate that females of *G. occidentalis* did not show significant differences in the preference for any odor (Figure 3). It seems they do not recognize *O. yothersi* volatiles as potential prey since the western predatory mite is an exotic predator in the tri-trophic system avocado-*O. yothersi*-predators, being necessary for further studies. However, postembryonic development and life table parameters of *G. occidentalis* obtained by us, indicates that *O. yothersi* is an excellent prey; therefore, we could develop the learning of this phytoseiid towards odors like *O. yothersi* kairomones and HIPVs, as well as predatory behavior. In that regard, the learning phenomenon has been studied in various predators such as phytoseiids, where Christiansen et al. (2016) found that *Amblyseius swirskii* Athias-Henriot (Acari: Phytoseiidae) is capable of learning in foraging predatory mites.

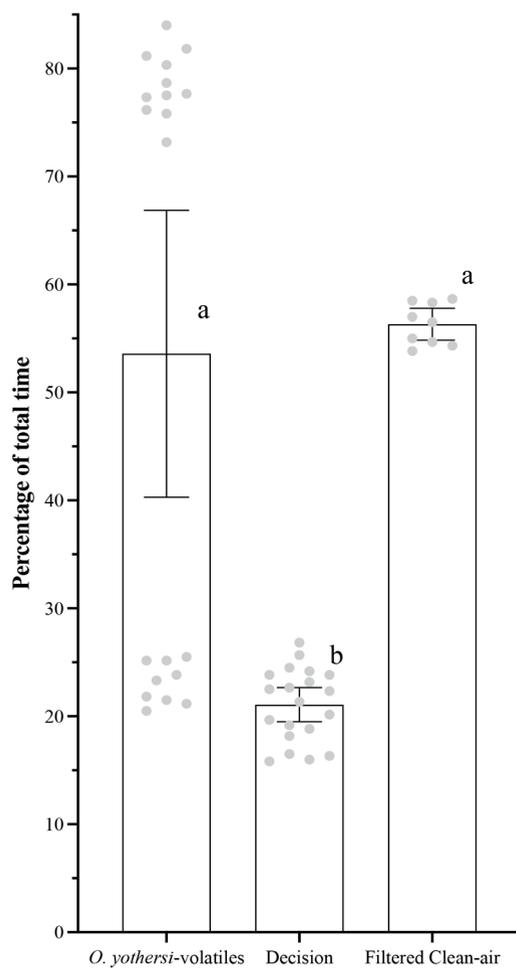
In addition, we offered synthetic compounds as odor sources to females of *G. occidentalis*. Our results indicate that *G. occidentalis* was attracted to 100 µg mL<sup>-1</sup> MeSA (Figure 4). Gadino et al. (2012a) reported that *Typhlodromus pyri* (Acari: Phytoseiidae) was significantly attracted to MeSA at doses 0.02, 0.2 and 20 µg. Similarly, Shastani et al. (2014) found that *P. persimilis* preferred synthetic MeSA at doses of 0.02-20 µg. However, *P. histrio* preferred MeSA concentration ranges 1-100 µg mL<sup>-1</sup>, while *O. pygmaea* showed attraction at 1 and 10 µg mL<sup>-1</sup> of synthetic MeSA (Rioja et al., 2018). Yet, when Shimoda (2010) offered synthetic MeSA (1 mg) vs. infested bean plants (*P. vulgaris* ‘Nagauzuramame’) with *T. urticae*, *Neoseiulus californicus* (Acari: Phytoseiidae) did not discriminate between these odor sources, showing that MeSA is an attractant for mite predators. Moreover, our results reveal that *G. occidentalis* spends more time visiting the ocimene zone at 100 µg mL<sup>-1</sup> (Figure 5) than other concentrations. In addition, Rioja et al. (2018) reported that *P. histrio* and *O. pygmaea* showed attraction to ocimene at 100 and 1000 µg mL<sup>-1</sup>, respectively.

**Table 4. Concentrations of volatile organic compounds collected from avocado (*Persea americana*) ‘Hass’ uninfested and *Oligonychus yothersi*-infested.**

Compound	Avocado shoots	
	Uninfested	Infested
	µg mL <sup>-1</sup>	
3-Hexanone	0.21	1.09
2-Hexanone	0.17	0.41
3-Hexanol	0.18	0.97
<i>cis</i> -3-Hexene-1-ol	nd	0.22
2,3,4-Trimethylhexane	0.27	0.29
α-Pinene	0.34	0.64
β-Pinene	0.31	0.63
β-Myrcene	nd	0.29
Cumene	0.11	0.15
D-Limonene	0.37	0.41
2-Propyl-1-pentanol	nd	0.23
β- <i>trans</i> -Ocimene	nd	3.63
β- <i>cis</i> -Ocimene	2.05	150.43
6-Ethyl-2-methyloctane	1.02	nd
β-Linalool	nd	17.09
(4 <i>E</i> ,6 <i>Z</i> )-2,6-Dimethyl-2,4,6-octatriene	nd	1.57
Methyl salicylate	nd	53.62
4,7-Dimethylundecane	nd	1.54
α-Farnesene	nd	22.07

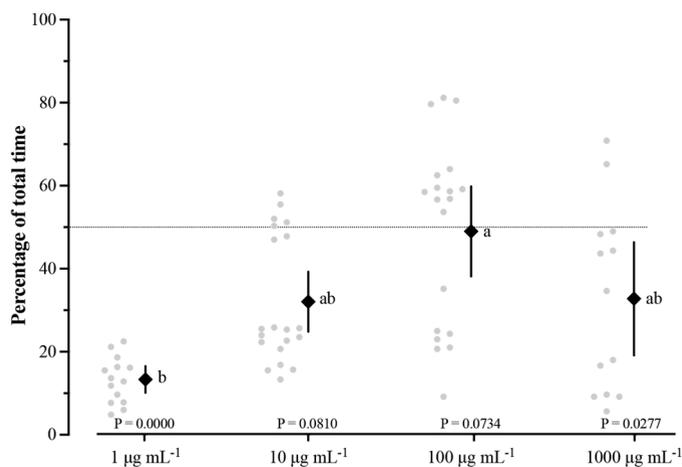
nd: Not detected.

**Figure 3. Olfactory responses of *Galendromus occidentalis* to volatiles emitted by *Oligonychus yothersi*.**



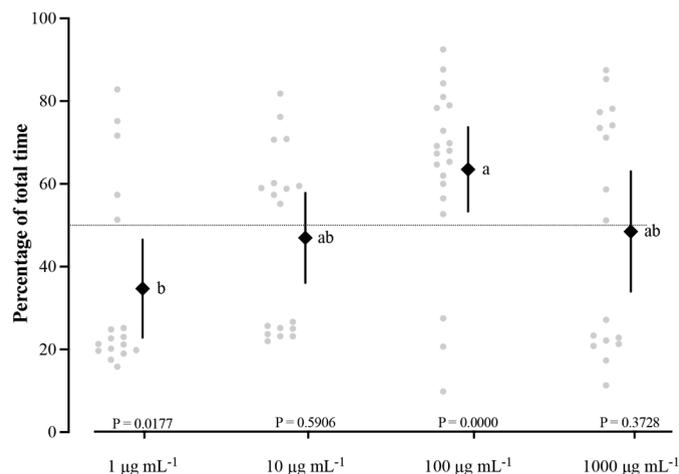
Mean spent time ( $\pm$  SD). Bars sharing a letter do not significantly differ according to the *U* Mann-Whitney test ( $P < 0.05$ ). A grey dot represents the spent time by a tested individual ( $N = 20$ ).

**Figure 4. Olfactory responses of *Galendromus occidentalis* to synthetic doses of methyl salicylate in Y-tube olfactometer.**



Mean spent time (black dot  $\pm$  CI<sub>95%</sub>). Means sharing a letter do not significantly differ according to Dunn's test ( $P < 0.05$ ). P-value at bottom indicate significant differences between stimulus and control according to the *U* Mann-Whitney test ( $P < 0.05$ ). Grey dots represent the spent time of each tested individual.

Figure 5. Olfactory responses of *Galendromus occidentalis* to synthetic ocimene in Y-tube olfactometer.



Mean spent time (black dot  $\pm$  CI<sub>95%</sub>). Means sharing a letter do not significantly differ according to Dunn's test ( $P < 0.05$ ). P-value at bottom indicate significant differences between stimulus and control according to the *U* Mann-Whitney test ( $P < 0.05$ ). Grey dots represent the spent time of each tested individual.

## CONCLUSIONS

Our results showed that *Galendromus occidentalis* completed its embryonic and postembryonic development on *Oligonychus yothersi* colonies, with higher survival rates and fertility at laboratory conditions. The avocado red mite had a positive effect on *G. occidentalis* fitness since we registered life table parameters within averages reported for *Galendromus* gender and a higher net reproductive rate. Hence, *O. yothersi* is an ideal prey for rearing *G. occidentalis*.

The herbivore-induced plant volatiles (HIPVs) released by *O. yothersi*-infested avocado shoots and *O. yothersi* volatiles did not attract females of *G. occidentalis*. However, *G. occidentalis* could distinguish concentrations of synthetic compounds as methyl salicylate and ocimene. Therefore, our findings hint at a partial rejection of the proposed hypothesis since *G. occidentalis* did not show attraction to HIPVs or *O. yothersi* volatiles. It is expected that the results of this study serve as a first step to understand the behavior of the western predatory mite and its evaluation as a biological agent on *O. yothersi*.

In addition, as a projection of this research, we consider evaluating the learning phenomenon in *G. occidentalis* using the same model (avocado – *O. yothersi*) study getting thus improved populations of phytoseiids in laboratory to release them in avocado orchards.

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