Incidence of eriophyid (Acariformes: Eriophyidae) and predatory mites (Parasitiformes: Phytoseiidae) in Florida citrus orchards under three different pest management programs

Emilie P. Demard
edemard@ufl.edu

University of Florida, Indian River Research and Education Center
Ismail Döker
Cukurova University
Jawwad A. Qureshi
University of Florida, Southwest Florida Research and Education Center

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Research Article

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Title Page

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Authors: Emilie P. Demard[1], Ismail Döker[2], Jawwad A. Qureshi[1,3]

[1]University of Florida, Indian River Research and Education Center, Fort Pierce, FL 34945, USA, edemard@ufl.edu. ORCID: 0000-0002-8260-7329.

[2]Cukurova University, Agricultural Faculty, Plant Protection Department, Acarology Laboratory, 01330 Adana, Turkey, idoker@cu.edu.tr ORCID: 0000-0002-1412-1554.

[3]University of Florida, Southwest Florida Research and Education Center, Immokalee, FL 34142, USA, jawwadq@ufl.edu. ORCID: 0000-0001-9076-4079.

*__Corresponding author__: Emilie P. Demard

Mailing address: University of Florida, Citrus Research and Education Center, Lake Alfred, FL 33850, USA.

E-mail addresses: edemard@ufl.edu

Abstract

The abundance and diversity of eriophyid and phytoseiid mites in south and central Florida were assessed in six citrus orchards under three different pest management systems, conventional, organic, and untreated. Tree canopy, ground cover, and leaf litter were sampled every two months in two groves each under conventional, organic, and untreated pest management from

April 2019 to February 2021. The citrus rust mite, *Phyllocoptruta oleivora* (Ashmead) was identified from 95-99% of the samples from study groves except 45% in one untreated orchard (n = 938). The pink citrus rust mite, *Aculops pelekassi* (Keifer) was present in organic and untreated orchards at 5% and 28%, respectively, but absent from conventional orchards (n = 134). Twenty-nine species of phytoseiid mites were identified from 1,778 specimens. Thirteen species were present in the canopy, fifteen in the ground cover, and eighteen in the leaf litter with some common species among these habitats. In the tree canopy, *Typhlodromalus peregrinus* (39%), *Euseius* spp. (25%), and *Iphiseiodes quadripilis* (19%) were the dominant species. *Typhlodromalus peregrinus* (43%), *Typhlodromips dentilis* (25%), and *Proprioseiopsis mexicanus* (13%) were the major species in the ground cover. Species richness was lower in organic orchards (3.0) compared to conventional and untreated orchards (5.0 and 4.7, respectively). In the leaf litter, *Amblyseius curiosus* (26%), *Proprioseiopsis carolinianus* (15%), *Chelaseius floridanus* (14%), and *A. tamatavensis* (12%) were the most common species. Shannon index was significantly higher in conventional orchards (1.45) compared to organic and untreated orchards (1.02 and 1.05, respectively). Evenness was also higher in conventional orchards (0.86) compared to organic and untreated (0.72 and 0.68, respectively). Finding of several phytoseiids in abundance across pest management programs suggest the need for identifying their role in pest suppression particularly mites.

Keywords: Citrus, Diversity, Biological control, Rust mites, Predatory mites, Ground cover.

Introduction

The citrus rust mite*,* *Phyllocoptruta oleivora* (Ashmead), and the pink citrus rust mite, *Aculops pelekassi* (Keifer), are economically important pests of Florida citrus (Childers and Achor 1999; Qureshi et al. 2021). Feeding damage on fruit leads to the death of epidermal cells resulting in lower fruit, quality specifically for the fresh market (Qureshi et al. 2021). For many decades, citrus pest management relied strongly on biological control along with applications of sulfur and petroleum oil for mite control (McCoy 1985; Qureshi and Stansly 2020). After the discovery of Huanglongbing (HLB) or citrus greening disease in 2005 (Halbert 2005), the increased use of insecticides for the control of the Asian citrus psyllid (ACP), *Diaphorina citri* Kuwayama, the vector of the causal bacterial agent of the disease, disrupted the biological control of several pests, including mites (Hoy 2011). Indeed, the use of insecticides, as well as copper-based fungicides to manage citrus canker caused by *Xanthomonas citri* ssp. *citri* (Hasse 1915), raises concerns about the resurgence of secondary pests and pest resistance (Childers 1994a, b; Childers and Selhime 1983; Hoy 2011). Since no cure has been found to overcome HLB, new production methods such as Citrus Under Protective Screen (CUPS) are developed to exclude the Asian citrus psyllid and produce disease-free citrus. CUPS structures are covered by a 0.3 mesh monofilament high-density polyethylene screen (Ferrarezi et al. 2017). Studies have shown that CUPS provides high-quality fresh fruit and better yields (Ferrarezi et al. 2019; Schumann and Waldo 2016; Schumann et al. 2021). However, the unique environmental conditions in CUPS favor the development of several pests including mites (Ferrarezi et al. 2017, Demard 2022). *Phyllocoptruta oleivora* and *Aculops pelekassi* are common pests in CUPS and open production systems (Demard 2022, Qureshi et al. 2021).

Predatory mites in the family Phytoseiidae (Acari: Parasitiformes) are important biological control agents of pest mites and small insects (McMurtry 1982). Studies in Alabama (Fadamiro et al. 2009), Guadeloupe (Mailloux et al. 2010), Tunisia (Sahraoui et al. 2016), Iran (Jalil and Maedeh 2012), and Syria (Barbar 2014) assessed the abundance and diversity of phytoseiids in the tree canopy or ground cover of citrus orchards. In Florida, Muma and Denmark (1970) and Muma (1975) sampled phytoseiid populations in the 1950s and 1960s to describe the species complex in citrus orchards. Childers and Denmark (2011) surveyed seven citrus orchards receiving reduced to no pesticide from 1994 to 1996 to compare phytoseiid abundance and distribution within the tree canopy and ground cover. Dooryard trees and commercial citrus trees were also sampled from 2009 to 2014 (Childers et al. 2022). However, none of these studies compared the effect of pest management programs on phytoseiid diversity and abundance. Several studies have shown the negative effects of insecticides on the field populations of insect predators, such as ladybeetles, although there is not much information on phytoseiids in Florida (Khan et al. 2014; Qureshi and Stansly 2007). Some recent studies have already reported lower densities and diversity of phytoseiids under conventional management practices (Sahraoui et al. 2016; Silva 2019; Szabó et al. 2014). Sahraoui et al. (2016) observed that species richness and abundance of phytoseiids were higher in organic citrus orchards than in conventional or IPM orchards. In apple orchards, phytoseiid species richness was lowest in conventionally managed orchards and highest in organic orchards while intermediate in IPM-managed and abandoned orchards (Szabó et al. 2014). Silva et al. (2019) noticed higher abundance and richness of predatory mites in abandoned vineyards than in organic and conventionally managed vineyards.

Besides pest management programs, ground cover management may also influence phytoseiid populations. The ground cover serves as a reservoir for natural enemies through the provision of alternative food, refugia, and breeding sites (Gravena et al. 1993; Liang and Huang 1994; Mailloux et al. 2010; Pina et al. 2012). Childers and Denmark (2011) observed higher phytoseiid populations in ground cover from March to April in Florida citrus orchards. They noticed that the abundance of *Typhlodromalus peregrinus* (Muma) was reduced by the absence of ground cover. The plant species composition of ground cover, as well as cultural practices, may also affect phytoseiid densities and species assemblage. Mailloux et al. (2010) found that reduced mowing practices and the use of *Neonotonia wightii* (Wight and Arn.) Lackey, as a cover crop improved phytoseiid densities and diversity in Guadeloupean citrus orchards. In addition, they observed lower species richness and densities in ground cover regularly sprayed with glyphosate and/or regularly mowed.

The specific objectives of this study were to (i) assess the influence of three pest management programs (conventional, organic, untreated) on the populations of eriophyid mites; (ii) evaluate the effect of pest management programs on the abundance, species composition and diversity of phytoseiid mites; (iii) compare the results to previous surveys of phytoseiids done in Florida citrus orchards; (iv) identify a dominant phytoseiid species in citrus orchards for use as a potential predator of pest mites in traditional open systems and CUPS (Citrus Under Protective Screen).

Materials and methods

Study sites

Six citrus orchards (two conventionally managed, two organic, and two untreated) in south and central Florida were sampled for eriophyid (rust mites) and phytoseiid mites (Table 1). The sites were sampled every two months from April 2019 to February 2021 except in April 2020 due to COVID-19 restrictions. Spray schedule and ground cover management practices are summarized in the Supplementary Material (Tables S1, S2, S3).

Table 1

Description of the six citrus orchards sampled from April 2019 to February 2021

Management	County	Geographic coordinates	Altitude (m)	Scion	Rootstock	Acreage	Age
Conventional	Saint Lucie	27°30'28.3"N 80°36'50.4"W	8	Ray Ruby Grapefruit	Sour Orange	8	8-10 years
	Indian River	27°35'23.8"N 80°37'11.5"W	9	Flame Grapefruit	NA	3	10-20 years
Organic	Lake	28°36'35.9"N 81°44'59.8"W	45	Red Grapefruit	Swingle citrumelo	3	23 years
	Lake	28°39'36.6"N 81°44'52.3"W	63	Navel	Citrange carrizo	8	21 years
Untreated	Indian River	27°39'17.0"N 80°27'49.6"W	7	Mix of varieties (Navel, Red Navel, Sweet Orange)	Sour Orange	3	6 years
	Saint Lucie	27°23'27.7"N 80°27'50.5"W	7	Page and Nova	US897	3	6 years

Sampling habitats and methods

Canopy

In each cardinal direction of the orchard, three trees from the border rows and three from the inner rows were randomly chosen for a total of 24 trees sampled per site to conduct sampling for eriophyids and phytoseiids. The canopy of a tree was divided into four quadrants (north, south, east, and west) and one leaf was randomly selected from each quadrant. The abaxial surface was examined for eriophyids with a magnifying hand lens (10X), for a total of 96 leaves (4 leaves x 24 trees) per site at each sampling point.

Two methods were used to sample phytoseiid populations: the dipping-washing method and the tap sampling method. For the dipping-washing method, four shoots with four to five leaves per tree were cut for a total of 96 shoots per orchard. The samples were dipped in a jar filled with 250 mL of 80% ethanol. The jar was shaken for about 30 seconds to dislodge the mites. The vegetative material was then retrieved from the jar using forceps and discarded. Shoots collected from the border and inner rows of the orchard were placed in two separate jars, which were returned to the laboratory for processing. One jar contained phytoseiids sampled from 12 trees (4 sides x 3 trees). To determine phytoseiid abundance in the trees, the tap sampling method developed by Qureshi and Stansly (2007) to quantify the abundance of the Asian citrus psyllid and other arthropods was used. The tap sample was conducted by tapping a randomly selected tree branch three times with a length of PVC pipe onto a blackboard. The number of phytoseiids falling on the blackboard was recorded. The same trees sampled for the dipping-washing method were used at one tap sample per tree. Twenty-four tap samples were conducted at each study site.

Ground cover

The dipping-washing method was used to process the samples of material collected from the ground cover. Four subsamples of ground cover, one from each side of the orchard, were collected. Ground cover samples consisted of unidentified low herbaceous plants (leaves, stems, and flowers when present) which were cut with pruning shears. For each subsample, a sufficient volume of the vegetative material was gathered in a 473-mL jar filled with 250 mL of 80% ethanol.

Leaf litter

Four leaf-litter sub-samples, one from each side of the orchard, were taken by collecting dried leaf material below a citrus tree and mixed. In each orchard, a sufficient volume of leaf litter was collected to fill up a 4-gallon capacity plastic bag. Phytoseiidae specimens were extracted from the vegetative material with a Berlese funnel (Collapsible Berlese funnel, BioQuip, CA, USA). One Berlese funnel per orchard was set up (six Berlese funnels total). Phytoseiidae specimens were collected in a Whirl-Pak bag (200 mL capacity) filled with 80% ethanol.

In a survey between 1994 and 1996 by Childers and Denmark (2011), 200 leaves per orchard were sampled. Because low populations of phytoseiids were observed in the last few years (C. C. Childers, personal communication), the number of samples in the canopy was increased in our study to enhance the likelihood of finding phytoseiids. However, it was not possible to increase the number of ground cover and leaf litter samples due to the amount of time involved in processing the samples. Additionally, most arthropods particularly pest mites, colonize the tree canopy where phytoseiids could be used for their control, and this justified more samples.

Species identification and counts

Five slides containing two to five eriophyid mites each were prepared for each sampling date and orchard. Specimens were mounted in Hoyer's medium, and the slides were dried on a hot plate (Thermo Scientific Cimarec) at 80°C for five to six hours. Eriophyids were examined with a phase-contrast microscope (Olympus® BX-41). The specimens of *P. oleivora* and *A. pelekassi* were identified using the presence/absence of a longitudinal furrow; the ornamentation on the prodorsal shield and the length of the setae located on the prodorsal shield as suggested by Childers and Achor (1999).

Phytoseiid mites from each sample were sorted from other mites and counted under a stereomicroscope (40X magnification). The total number of phytoseiids (females, males, and immatures) was reported. Identification keys mainly rely on adult females for morphological identification, and therefore those were mounted. If the total number of phytoseiids exceeded 60 individuals per sample, then only 30% of the total count was sub-sampled and mounted. Female specimens were placed in vials containing 60% lactic acid overnight. They were then mounted on slides in Hoyer's medium and the slides were dried on a slide warmer (Premiere® XH-2004) at 50°C for five days. Phytoseiids were identified under a phase-contrast microscope (Olympus® BX-41) by using the generic classification of Chant and McMurtry (2007). Specimens were deposited in the Acarology Laboratory, Department of Plant Protection, Cukurova University, Adana, Turkey and the Indian River Research and Education Center, University of Florida, Fort Pierce, Florida, USA.

Data analysis

Data were analyzed with a generalized linear mixed model procedure for repeated measures as implemented in SAS PROC GLIMMIX (SAS/STAT 15.1; SAS Institute, Cary, NC) with a Poisson distribution function. When overdispersion was detected ($\chi 2/\text{df} > 1$), a negative binomial distribution was used instead of a Poisson.

For the seasonal abundance of rust mites and phytoseiids, mean monthly counts of mites were calculated per management program (conventional, organic, untreated), cardinal direction in the orchard (north, south, east, west), tree location in the orchard (border and inner rows), tree canopy quadrant (north, south, east, west), and sampling month. Management program, tree location, cardinal direction in the orchard, and sampling month were considered as fixed effects. Orchard was considered as a random effect. For the rust mites, data were compared among all three management programs first and then between organic and untreated orchards only because numbers in the conventional orchards were very low (means < 0.45 mites/lens field), and there were no significant effects when included in the analysis. Data and analyses are only presented for untreated and organic programs. Since there was no significant effect of the tree quadrant and its interaction with other effects ($P > 0.59$), we simplified the model by eliminating this factor.

To determine the abundance of Phytoseiidae from the jar samples, the counts of mites were averaged per management program, orchard, habitat (canopy, ground cover, leaf litter), and sampling month. Management, tree location in the orchard (border and inner rows), and sampling months were treated as fixed effects. Orchard was considered as a random effect.

To compare species diversity among pest management programs, four indices were estimated:

Shannon Index H’ (Shannon and Weaver 1949):

$$H' = -\sum_{i=1}^{s} p_i ln\, p_i =-\sum_{i=1}^{s}\left[(\frac{n_i}{N}) \times \ln(\frac{n_i}{N})\right] \tag{1}$$

Where *s* is the number of species in the community, p_i is the proportion of individuals (n_i/*N*) in the *i*th species, n_i is the number of individuals in the *i*th species, and *N* is the total number of individuals for all species.

Evenness J:

$$J = \frac{H'}{\ln s} \tag{2}$$

Simpson's index D (Simpson 1949):

$$\mathrm{D} = \sum_{i=1}^{s}(p_i)^2 = \sum(\frac{n_i}{N})^2 = \sum_{i=1}^{s}\frac{n_i(n_i-1)}{N\,(N-1)} \tag{3}$$

Species richness R, which is the total number of species present in each sample.

Evenness and Simpson's index values vary from 0 to 1. An evenness value of 0 means that only one species is present in the site, while a value of 1 means that the site reached complete evenness. The Simpson's index is an index of dominance, the closer the value to 0, the higher the diversity. Shannon index values range from 0 to 5 and are usually between 1.5 and 3.5. This index is sensitive to the number of species in a sample. The more unequal the abundance of species, the smaller the index.

The abundance was also calculated for the total females mounted. For some sampling dates, only one species was present in a given sample, thus the total number of species per sampling date was not enough to calculate diversity indices. Therefore, two to four sampling dates were pooled per growing season and gathered under the variable semester. The count of each species was summed per management, grove, and semester. Management program was considered a fixed effect and semester as a random effect.

Meteorological conditions were obtained using the Florida Automated Weather Network (FWAN) data stations available at https://fawn.ifas.ufl.edu/. Temperatures were recorded with a Bandgap sensor and relative humidity with a Capacitive sensor (Campbell Scientific® Model CS215). Precipitation was measured with a typing bucket rain gauge Model H-340. The maximum, minimum, and average temperatures, precipitations, and relative humidity were averaged by month. Weather data are presented in the Supplementary Material (Fig. S1 and S2).

Results

Eriophyid population dynamics and distribution

The interaction month × management program was significant in the analysis of data on rust mites from organic and untreated programs ($F_{10,14} = 13.91$, $P < 0.0001$). Rust mite numbers were significantly higher in untreated compared to organic orchards in April 2019 (mean = 0.5, 95%, CI 0.2-1.1, mean = 0.05, 95% CI 0.01-0.2, respectively) and June 2019 (mean = 3.1, 95% CI 1.6-6.3, mean = 0.06, 95% CI 0.01-0.2, respectively) (Fig. 1). On the other hand, counts of rust mites were higher in organic compared to untreated orchards in August 2019 (mean = 1.1, 95% CI 0.5-2.2, mean = 0.4, 95% CI 0.2-0.8, respectively), December 2019 (mean = 3.9, 95% CI 2.0-7.7, mean = 1.2, 95% CI 0.6-2.4, respectively), and October 2020 (mean = 1.1, 95% CI 0.5-2.3, mean = 0.03, 95% CI 0.003-0.2, respectively). Even though conventional orchards were not included in the analysis, the abundance of rust mites was very low throughout the two years of study, with the highest mean reaching 0.45 mites per hand lens in August 2020.

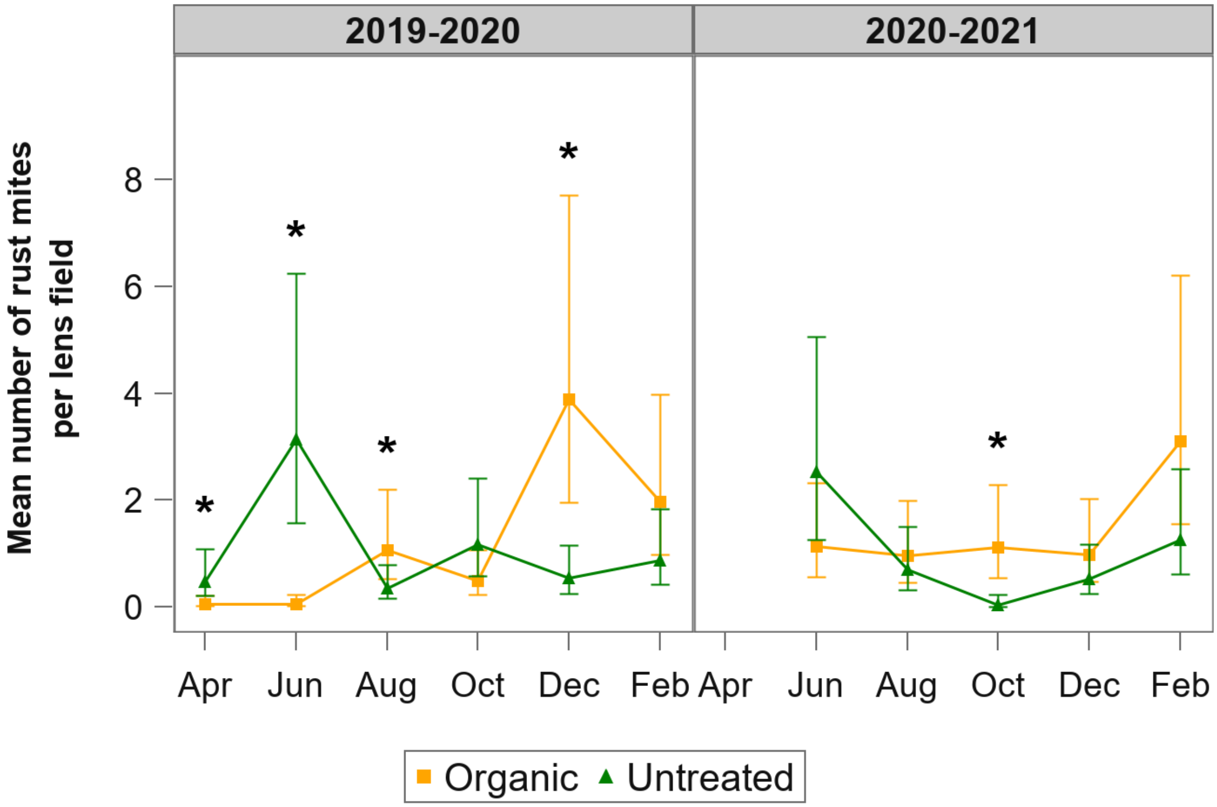

Fig. 1 Mean number (± CI) of rust mites per lens field. Asterisks indicate a significant difference between the two pest management programs ($P < 0.05$)

The interaction tree location in the orchard × management program was not significant ($F_{1,14} = 4.01$, $P = 0.065$), but we consider this needed to be further evaluated. There was no significant difference in rust mites counts between border and inner rows in organic orchards (mean = 0.8, 95% CI 0.4-1.4, mean = 0.7, 95% CI 0.4-1.3, respectively). Nevertheless, rust mites were significantly more numerous in border rows compared to inner rows in untreated orchards (mean = 0.9, 95% CI 0.5-1.7, mean = 0.5, 95% CI 0.2-0.9, respectively) (Fig. 2).

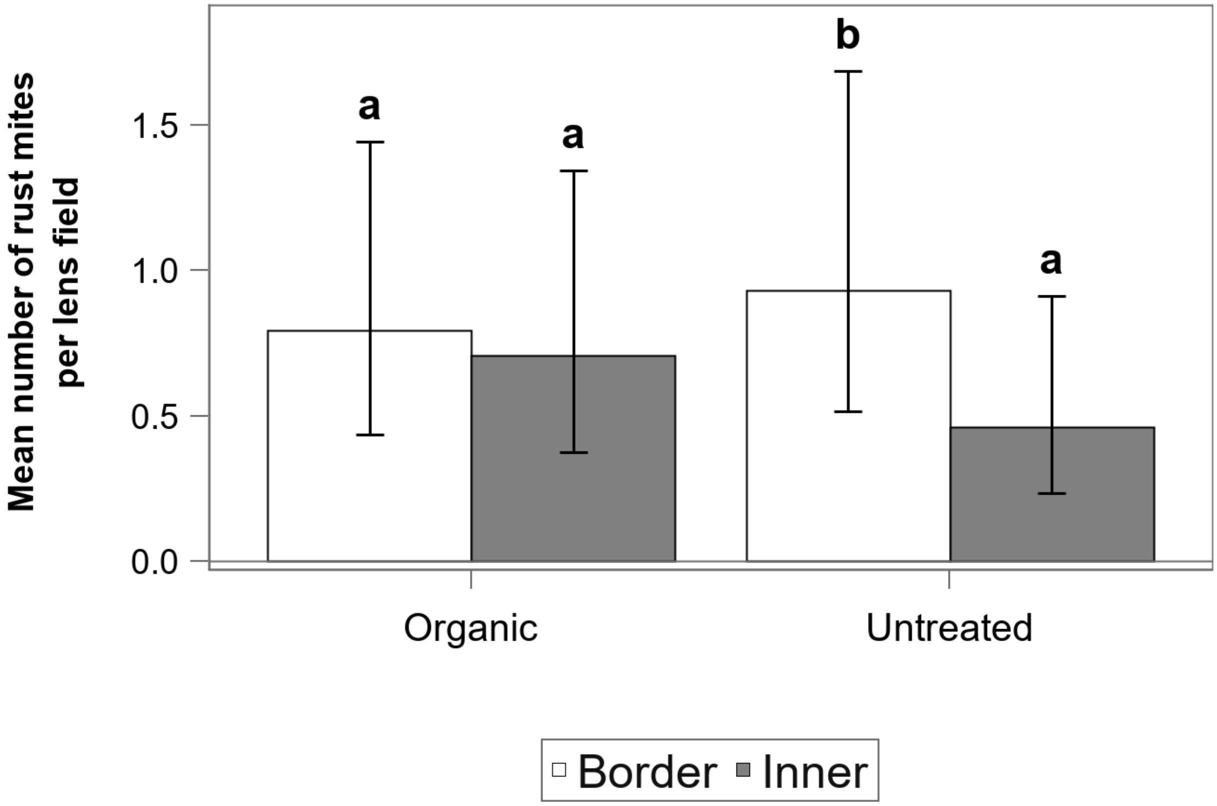

Fig. 2 Mean number (± CI) of rust mites per lens field for border and inner rows of the blocks depending on the pest management program. Different letters between the border and inner rows within the orchard management program indicate a significant difference ($P < 0.05$)

Eriophyid species assemblage and abundance

A total of 1,072 specimens of rust mites were mounted during the study (Table 2). Of these, 88% were *P. oleivora,* and 12% were *A. pelekassi*. In conventional and organic orchards, *P. oleivora* averaged 99% and 95% while the rest (< 5%) were *A. pelekassi*. In the untreated orchards, *A. pelekassi* was dominant in one orchard (55% *A. pelekassi* compared to 45% *P. oleivora*) but in the second untreated orchard, *P. oleivora* represented 98% of the total sample.

Table 2

Total counts and relative abundance of rust mite species mounted on slides in citrus orchards with different pest management programs [conventional, organic, untreated]

	P. oleivora		*A. pelekassi*		
Pest management	Counts	Relative abundance (%)	Counts	Relative abundance (%)	**Total counts**
Conventional	281	99.6	1	0.4	**282**
Organic	352	95.4	17	4.6	**369**
Untreated	305	72.4	116	27.6	**421**
Total	938	-	134	-	**1,072**

Phytoseiid population dynamics and distribution

Tap sample method

Tree location, cardinal direction in the grove, and their interactions with each other were not significant ($P > 0.13$). The interaction month × management program was significant ($F_{20,240} = 6.09$, $P < 0.0001$). Significantly higher numbers of phytoseiids were observed in organic (mean = 5.2, 95% CI 3.5-7.5) and untreated (mean = 3.9, 95% CI 2.6-5.7) orchards compared to conventional orchards (mean=1.5, 95% CI 0.9-2.4), with no difference between the former two in April 2019 (Fig. 3). However, significantly more phytoseiids in conventional orchards compared to organic and untreated orchards were observed in December 2019 (mean = 2.4, 95% CI 1.5-3.8, mean = 0.3, 95% CI 0.04-1.8, mean = 1.2, 95% CI 0.7-1.9, respectively), February 2020 (mean = 8.8, 95% CI 6.1-12.8, mean = 3.5, 95% CI 2.3-5.3, mean = 2.3, 95% CI 1.5-3.5, respectively), December 2020 (mean = 3.0, 95% CI 1.7-3.8, mean = 0.6, 95% CI 0.1-3.2, mean = 1.1, 95% CI 0.7-1.9, respectively), and February 2021 (mean = 2.6, 95% CI 1.7-3.8, mean = 1.2, 95% CI 0.7-1.9, mean = 1.1, 95% CI 0.7-1.8, respectively).

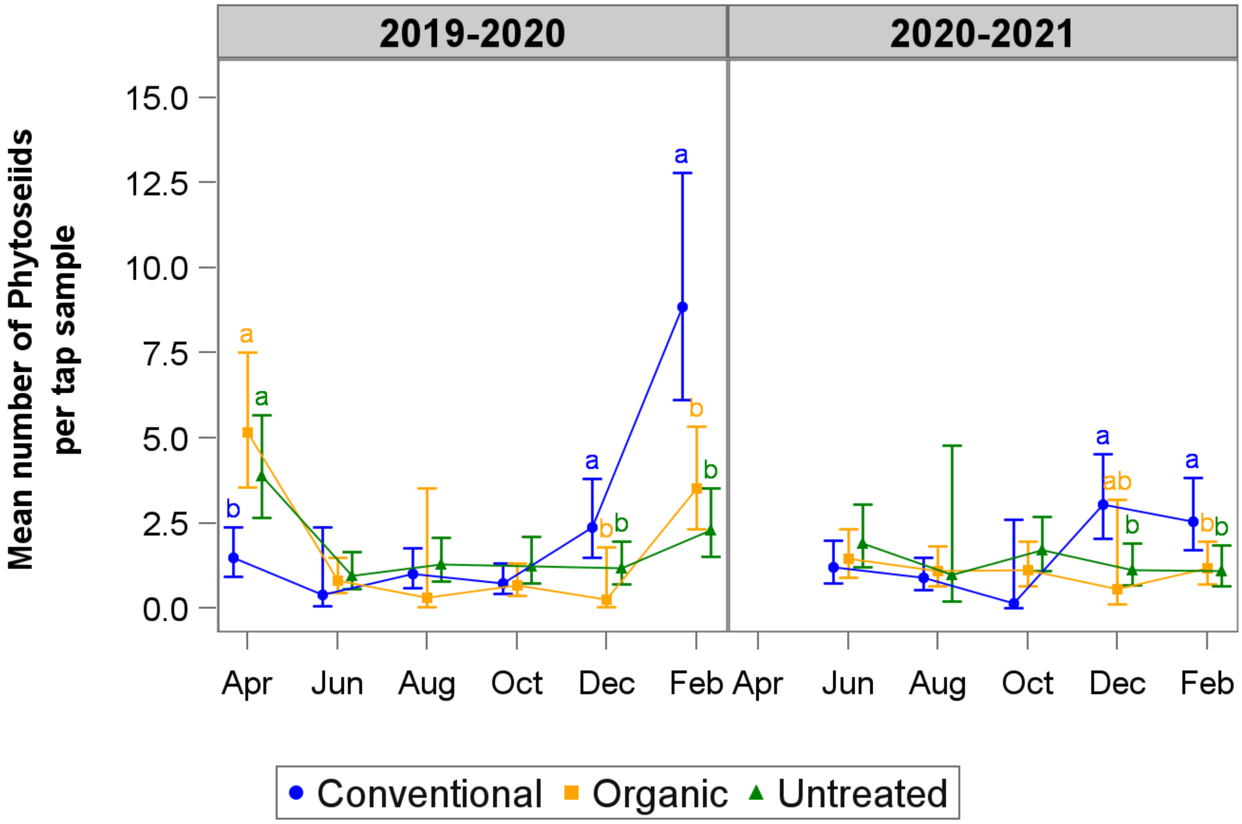

Fig. 3 Mean number (± CI) of adult and immature phytoseiids in the canopy using the tap sample method (24 tap samples/orchard). Different letters for a particular date indicate a significant difference between the three pest management programs [convention, organic, untreated] ($P < 0.05$)

Dipping-washing method

Canopy. Tree location did not affect the mean abundance of phytoseiids ($F_{1,3} = 0.68$, $P = 0.47$). The interaction month × management program was significant ($F_{20,60} = 7.21$, $P < 0.0001$). There were significantly more phytoseiids in organic compared to untreated and conventional orchards in April 2019 (mean = 52.1, 95% CI 26.6-101.9, mean = 14.0, 95% CI 6.8-28.5, mean = 7.4, 95% CI 3.5-15, respectively) and October 2020 (mean = 6.0, 95% CI 2.8-13.0, mean = 20.8, 95% CI 10.4-41.5, mean = 17.2, 95% CI 8.5-34.4, respectively) (Fig. 4). Other than April 2019 and October 2020, there were no differences in phytoseiid abundance between organic and untreated orchards. Phytoseiids were significantly more numerous in conventional compared to organic and untreated orchards in August 2019 (mean = 7.4, 95% CI 3.5-15.7, mean = 5.1, 95% CI 2.2-11.4, mean = 5.1, 95% CI 2.3-11.5, respectively), December 2019 (mean = 79.0, 95% CI 40.2-133.2, mean = 10.2, 95% CI 12.5-49.6, mean = 9.8, 95% CI 4.7-20.1, respectively), February 2020 (mean = 108.6, 95% CI 56.1-209.9, mean = 24.9, 95% CI, 12.5-49.6, mean = 26.1, 95% CI 13.2-51.7, respectively), December 2020 (mean = 74.4, 95% CI 38.3-144.3, mean = 28.0, 95% CI 14.2-55.3, mean = 11.4, 95% CI 5.5-23.5, respectively), and February 2021 (mean = 79.1, 95% CI 40.8-153.3, mean = 21.9, 95% CI 11.0-43.5 mean = 11.5, 95% CI 5.6-23.7, respectively).

Ground cover. The interaction month × management program was significant ($F_{20,30} = 5.17$, $P < 0.0001$). Significantly more phytoseiids were observed in conventional than in untreated orchards in April 2019 (mean = 28.5, 95% CI 11.9-68.2, mean = 1.4, 95% CI 0.3-6.2, respectively) (Fig. 4), with no difference between the organic and conventional orchards. Phytoseiids were significantly more abundant in organic and untreated compared to conventional orchards in June 2020 (mean = 59.4, 95% CI 24.7-143.0, mean = 17.8, 95% CI 7.2-44.2, mean = 3.0, 95% CI 0.9-9.7, respectively) and in organic compared to conventional orchards in October 2020 (mean = 16.5, 95% CI 6.7-40.9, mean = 2.9, 95% CI 0.9-9.5, respectively). In February 2021, significantly more phytoseiids were in conventional than in organic and untreated orchards (mean = 44.9, 95% CI 19.0-106.1, mean = 3.7, 95% CI 1.2-10.9, mean = 0.5, 95% CI 0.05-4.5, respectively).

Leaf litter. The interaction month × management program was significant ($F_{20,30} = 5.17$, $P < 0.0001$). In June 2019, phytoseiids were significantly more numerous in the untreated orchards compared to the conventional and organic orchards (mean = 16.1, 95% CI 6.1-42.4, mean = 1.4, 95% CI 0.3-6.1, mean = 2.7, 95% CI 0.7-9.8, respectively) (Fig. 4). In December 2019, organic orchards had more phytoseiids than conventional and untreated orchards (mean = 77.1, 95% CI, 31.1-191.1, mean = 4.6, 95% CI 1.49-14.2, mean = 9.7 95% CI 3.6-27.6, respectively). In February 2020, phytoseiids were significantly more numerous in organic orchards compared to conventional orchards but not significantly different from untreated orchards (mean = 77.6, 95% CI 30.9-200.9, mean = 17.5, 95% CI 6.7-45.4, respectively). There were no significant differences in phytoseiid abundance among orchard management from June 2020 to February 2021 as population levels were low.

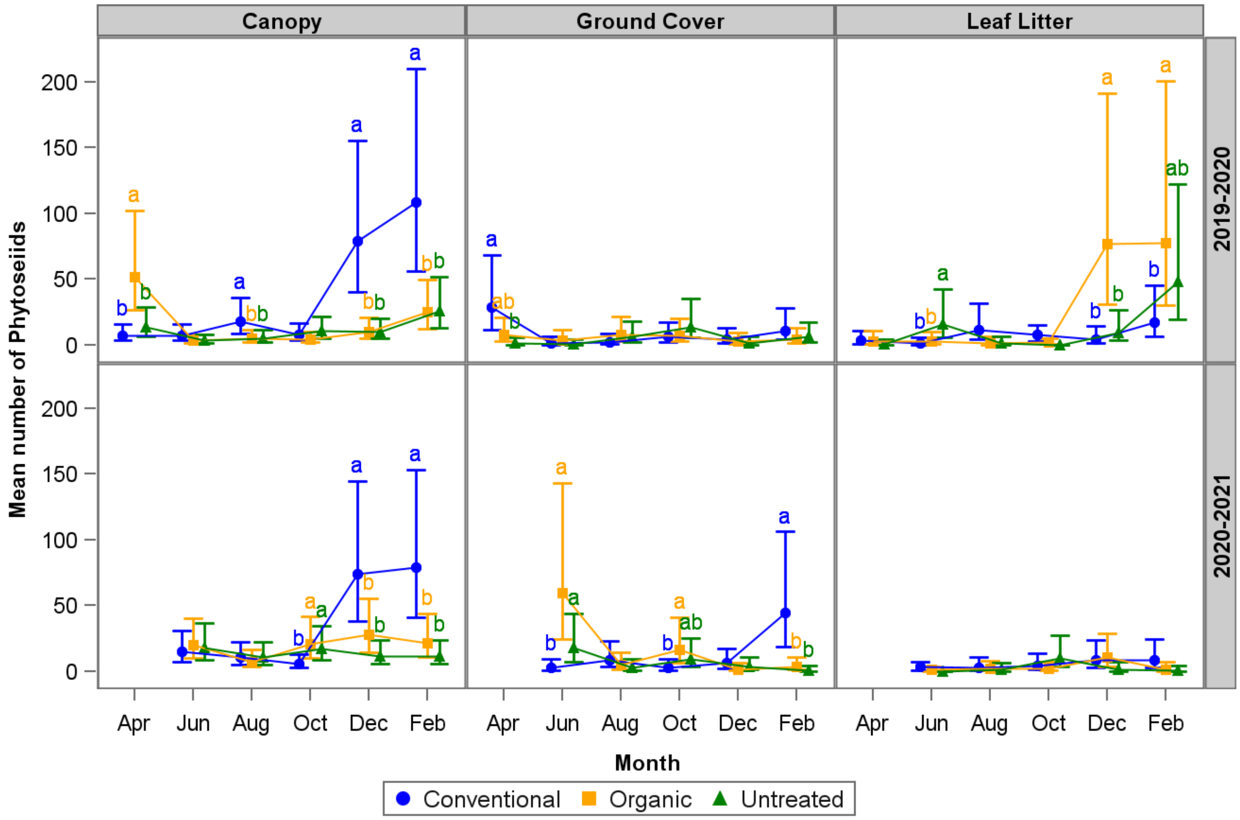

Fig. 4 Mean number (± CI) of adult and immature phytoseiids per sample using the dipping-washing method. For the ground cover and leaf litter, 4 sub-samples were taken per orchard while for the canopy 96 shoots of 4 leaves were sampled per orchard. Different letters for a particular date indicate a significant difference between the three pest management programs [conventional, organic, untreated] ($P < 0.05$)

Phytoseiid species assemblage and abundance

A total of 1,778 female specimens of at least 29 phytoseiid species were mounted and identified from 6 orchards (Table 3). *Euseius* spp., *Neoseiulus* spp. and *Proprioseiopsis* spp. were identified at the genus level, and the remaining 26 at the species level. Most *Euseius* specimens sampled in this study were identified as *Euseius mesembrinus* (Dean). However, small morphological differences were noticed among specimens, and we believe that a complex of at least two species was collected. Further examination of type materials is needed to confirm species names for the undetermined species.

Table 3

Phytoseiid species identified from the six citrus orchards surveyed in Florida from 2019 to 2021

Scientific name	Abundance
Amblyseius aerialis (Muma)	14
Amblyseius curiosus (Chant and Baker)	101
Amblyseius herbicolus (Chant)	1
Amblyseius largoensis (Muma)	21
Amblyseius obtusus (Koch)	3
Amblyseius swirskii Athias-Henriot	2
Amblyseius tamatavensis Blommers	159
Chelaseius floridanus (Muma)	50
Euseius spp.	276
Galendromus (Galendromus) floridanus (Muma)	1
Iphiseiodes quadripilis (Banks)	209
Neoseiulus gracilis (Muma)	21
Neoseiulus hexaporus Döker, Demard, Bolton and Qureshi	4
Neoseiulus longispinosus (Evans)	1
Neoseiulus marinellus (Muma)	11
Neoseiulus mumai (Denmark)	2
Neoseiulus planatus (Muma)	1
Neoseiulus spp.	3
Phytoscutus sexpilis Muma	3
Proprioseiopsis carolinianus (Muma, Metz and Farrier)	57
Proprioseiopsis citri (Muma)	43
Proprioseiopsis iphiformis (Muma)	4
Proprioseiopsis mexicanus (Garman)	64
Proprioseiopsis spp.	9

Proprioseius meridionalis Chant	12
Tenorioseius gracilisetae (Muma)	5
Typhlodromalus peregrinus (Muma)	566
Typhlodromina subtropica Muma and Denmark, 1969	33
Typhlodromips dentilis (DeLeon)	102
Total	**1,778**

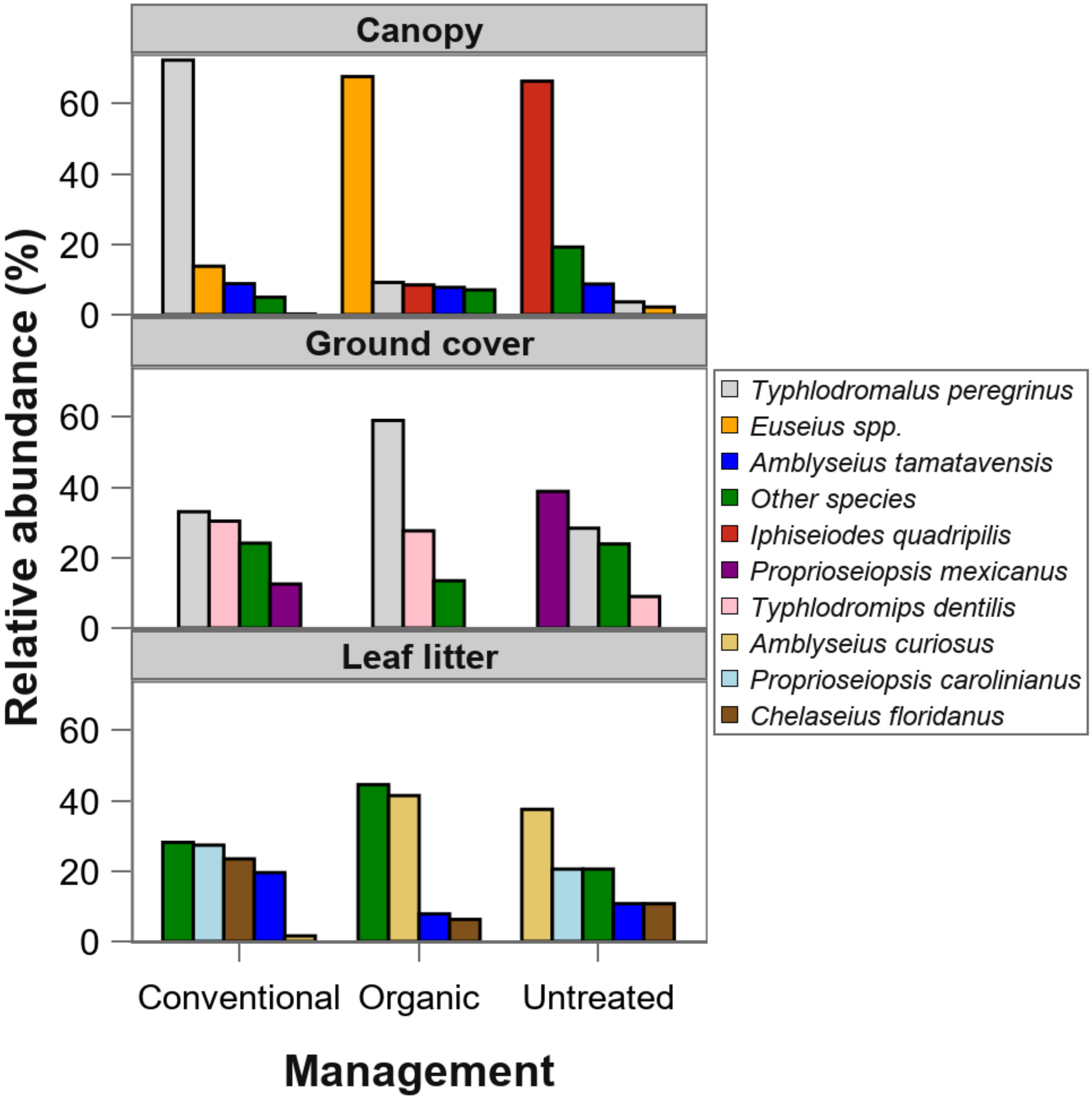

Fig. 5 Relative abundance (% of total mounted) of female phytoseiid species identified from three habitats in conventional, organic, and untreated orchards. Species with less than 40 individuals were combined under the group "other species"

From the canopy, 1,100 specimens from 13 species were mounted and identified. *Typhlodromalus peregrinus* (n = 430, 39%), *Euseius* spp. (n = 271, 25%), *Iphiseiodes quadripilis* (Banks) (n = 207, 19%) were the three most abundant species. *Typhlodromalus peregrinus* was the dominant species in conventional orchards (n = 394, 73%), while *Euseius* spp. and *I. quadripilis* were dominant in organic and untreated orchards, respectively (n = 191, 68% and n = 183, 67%) (Fig. 5). *Typhlodromalus peregrinus* numbers increased in December and February for the two sampling seasons. High numbers of *A. tamatavensis* were also observed in December 2020 in conventional orchards. *Iphiseoides quadripilis* and *Euseius* spp. densities increased slightly later in February and April. However, we could not confirm the trend for April 2020 since sampling was not conducted due to the pandemic (Fig. 6).

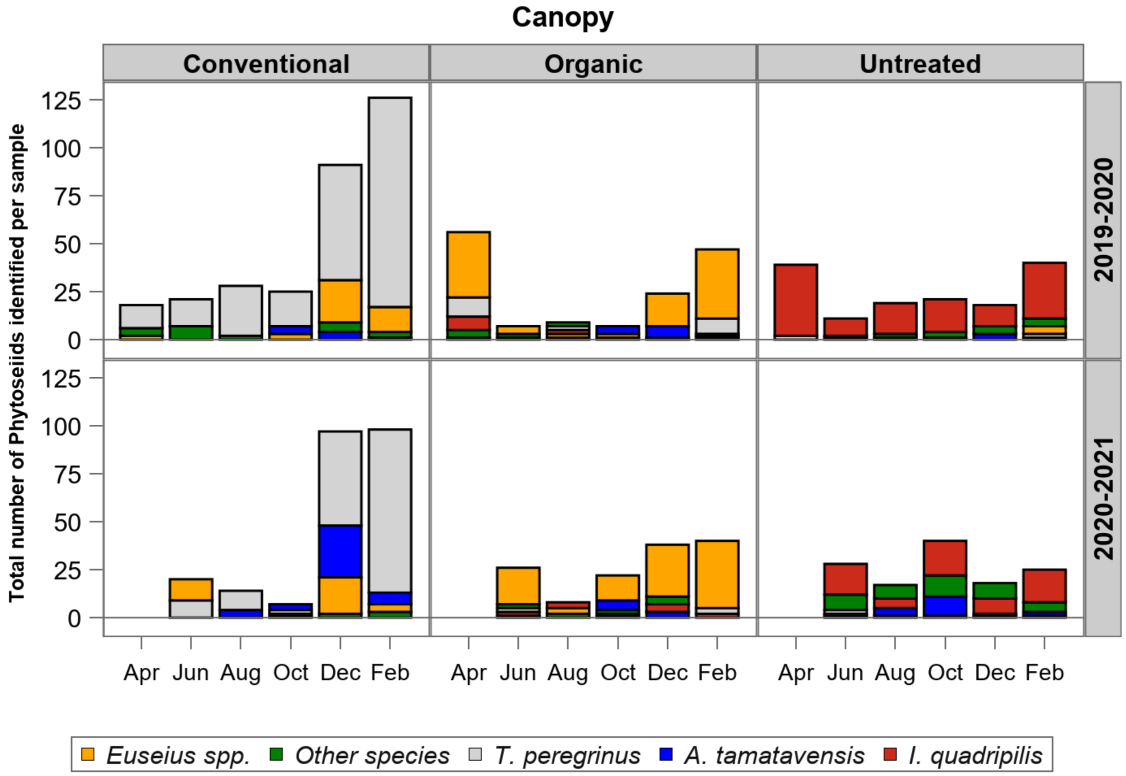

Fig. 6 Total number of phytoseiids mounted on slides from canopy samples (96 shoots of 4 leaves per orchard) under three pest management programs [conventional, organic, untreated]. Species with less than 200 individuals sampled per year were combined under the group “other species”

From ground cover, 313 female specimens of 15 species were mounted and identified. *Typhlodromalus peregrinus* (n = 135, 43%), *Typhlodromips dentilis* (De Leon) (n = 77, 24%), and *Proprioseiopsis mexicanus* (Garman) (n = 40, 13%) were the most abundant species. *Typhlodromalus peregrinus* and *T. dentilis* were dominant species in conventional orchards (n = 37, 33% and n = 34, 30%, respectively) whereas *P. mexicanus* and *T. peregrinus* were dominant in untreated orchards (n = 26, 39% and n = 19, 28%, respectively). In organic orchards, *T. peregrinus* was the most abundant species (n = 79, 59%) (Fig. 5). In conventional orchards, *T.*

peregrinus population increased in April 2019 even though no sampling was done in April 2020 to confirm this trend (Fig. 7). On the other side, high *T. dentilis* numbers were observed in February 2021. In June 2021, increasing counts of *T. peregrinus* and *P. mexicanus* are noticed in organic and untreated orchards, respectively.

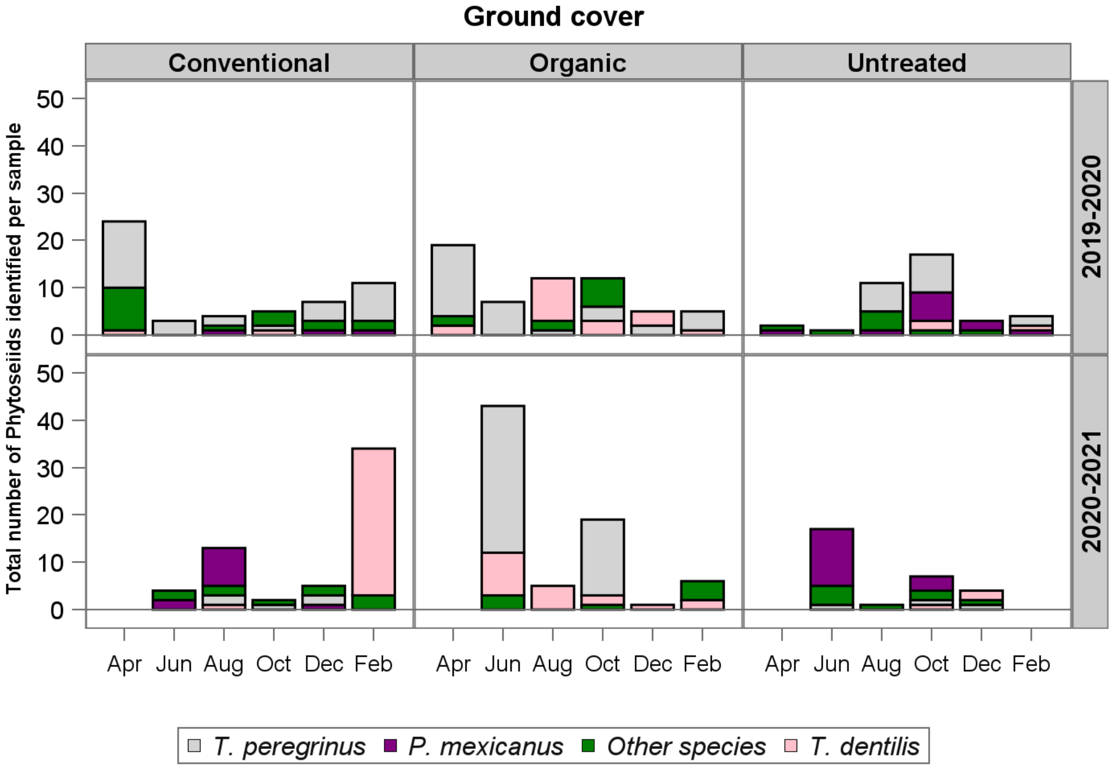

Fig. 7 Total number of phytoseiids mounted on slides from ground cover samples (4 sub-samples per orchard) under three pest management programs [conventional, organic, untreated]. Species with less than 40 individuals sampled per year were combined under the group “other species”

From leaf litter, 365 specimens of 18 species were mounted and identified. *Amblyseius curiosus* (Chant and Baker) (n = 97, 27%)*, Proprioseiopsis carolinianus* (Muma, Metz and Farrier) (n = 57, 16%)*, Chelaseius floridanus* (Muma) (n = 50, 14%)*,* and *Amblyseius tamatavensis* Blommers (n = 47, 13%) were the most common species. Contrary to the canopy and ground cover, species abundance was more even in the leaf litter. *Amblyseius curiosus* were commonly found in organic and untreated orchards (n = 53, 42% and n = 42, 38%, respectively) but scarce in conventional orchards (n = 2, < 2%). *Proprioseiopsis carolinianus* was commonly encountered in conventional and untreated orchards (n= 35, 27% and n = 22, 20%, respectively) but absent from organic orchards. *Chelaseius floridanus* and *A. tamatavensis* were common in conventional orchards (n = 30, 23% and n = 25, 20%, respectively) but were less abundant in organic (n = 8, 6% and n = 10, 8% n= 12, respectively) and untreated orchards (n = 12, 11% for both species) (Fig. 5). *Amblyseius curiosus* peaked in February 2019 in organic and untreated orchards although the trend was not observed the second year of sampling (Fig. 8). Higher numbers of *P. carolinianus* were observed in August 2019 and October 2020 in conventional and untreated orchards, respectively. The peak of phytoseiids observed in organic orchards in December 2019 includes mainly *N. gracilis* (n=17, n=38%) and *A. curiosus* (n=10, 22%) (Fig. 8). Finally, higher densities of *A. tamatavensis* are noticed in February 2020 and 2021 in conventional orchards.

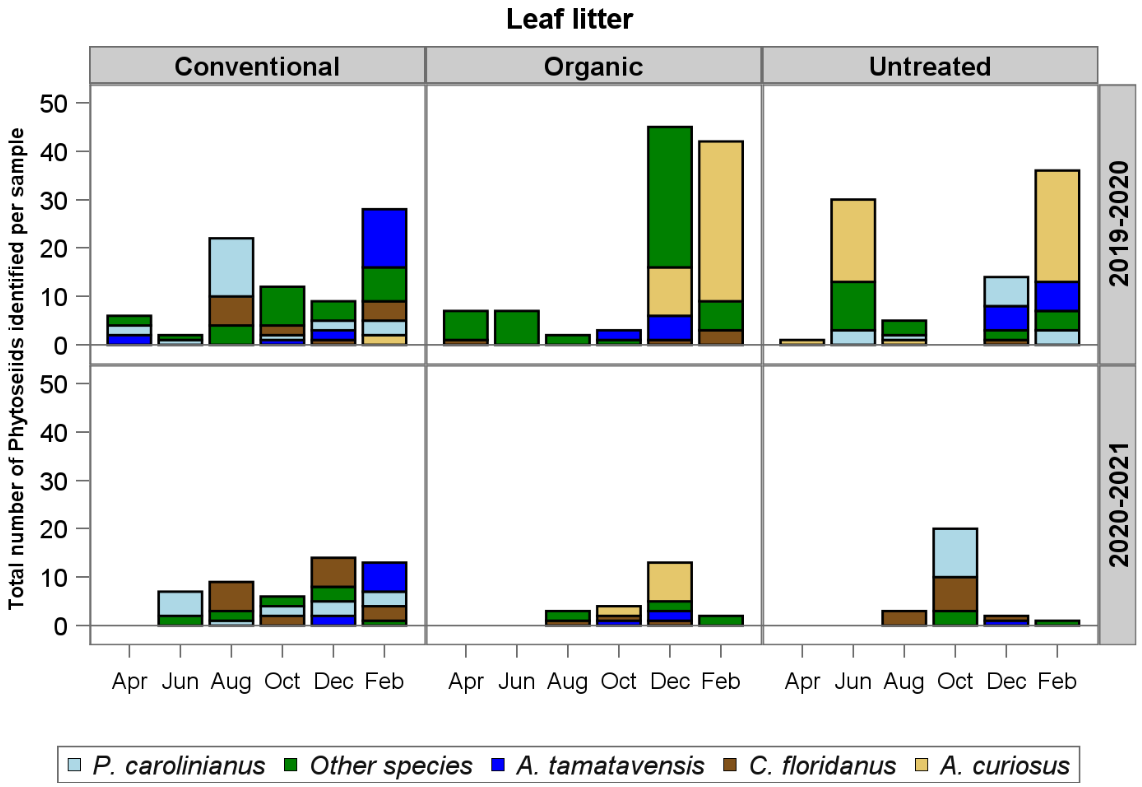

Fig. 8 Total number of phytoseiids mounted on slides from leaf litter samples (4 sub-samples per orchard) under three pest management programs [conventional, organic, untreated]. Species with less than 40 individuals sampled per year were combined under the group "other species"

Phytoseiid diversity indices

In the canopy, the evenness, richness, Shannon, and Simpson's indices were not significantly different among management programs ($P > 0.15$) (Table 4). Abundance was significantly higher in conventional orchards ($F_{2,13} = 4.42$, $P = 0.03$).

Table 4

Female abundance, evenness, richness, Shannon index, and Simpson's index for phytoseiids collected from orchards under three citrus pest management programs [conventional, organic, untreated] and in three habitats [canopy, ground cover, leaf litter]. Means in the same row followed by different letters are significantly different (P < 0.05)

			Management				
Habitats	Parameters	Conventional	Organic	Untreated	F	Df	P-value
Canopy	Abundance	90.83 [61.31-120.36] a	47.33 [17.81-76.86] b	46.00 [16.48-75.53] b	4.61	2, 13	0.03
	Evenness	0.60 [0.37-0.82] a	0.56 [0.34-0.79] a	0.60 [0.38-0.83] a	0.04	2, 13	0.96
	Richness	3.17 [1.56-4.77] a	4.67 [3.06-6.27] a	5.34 [3.73-6.93] a	2.24	2, 13	0.15
	Shannon Index	0.66 [0.20-1.12] a	0.95 [0.49-1.41] a	0.97 [0.51-1.42] a	0.65	2, 13	0.54
	Simpson's Index	0.61 [0.39-0.83] a	0.56 [0.33-0.78] a	0.53 [0.31-0.75] a	0.16	2, 13	0.85
Ground cover	Abundance	18.67 [9.19-28.14] a	22.33 [12.85-31.81] a	11.17 [1.69-20.64] a	1.68	2, 13	0.22
	Evenness	0.70 [0.56-0.83] a	0.79 [0.64-0.93] a	0.84 [0.71-0.97] a	1.29	2, 12	0.31
	Richness	5.0 [3.84-6.15] a	3.0 [1.84-4.16] b	4.7 [3.51-5.82] a	5.64	2,13	0.02
	Shannon Index	1.12 [0.85-1.40] a	0.94 [0.64-1.25] a	1.22 [0.95-1.49] a	1.1	2,12	0.36
	Simpson's Index	0.44 [0.31-0.57] a	0.44 [0.30-0.58] a	0.37 [0.25-0.50] a	0.41	2,12	0.67
Leaf litter	Abundance	21.33 [5.52-37.15] a	21.33 [5.52-37.15] a	18.67 [2.84-34.48] a	0.1	2, 13	0.9
	Evenness	0.86 [0.78-0.95] a	0.72 [0.63-0.82] b	0.68 [0.58-0.77] b	5.89	2, 11	0.02
	Richness	5.50 [3.64-7.35] a	4.00 [2.14-5.86] a	4.17 [2.31-6.02] a	2.06	2, 13	0.17
	Shannon Index	1.45 [1.22-1.69] a	1.02 [0.78-1.27] b	1.05 [0.80-1.29] b	12.3	2, 11	0.002
	Simpson's Index	0.28 [0.20-0.36] b	0.46 [0.37-0.55] a	0.48 [0.39-0.57] a	10.8	2, 11	0.003

In the ground cover, the abundance, evenness, Shannon, and Simpson's indices were not significantly different among pest management programs ($P > 0.22$), but the richness was significantly lower in organic orchards ($F_{2,13} = 5.64$, $P = 0.02$) (Table 4).

In the leaf litter, there was no significant difference in abundance and richness. Evenness, Shannon, and Simpson's indices were significantly different across the pest management programs ($F_{2,11} = 5.89$, $P = 0.02$; $F_{2,11} = 12.34$, $P = 0.002$; $F_{2,11} = 10.78$, $P = 0.003$, respectively) (Table 4).

Discussion

Eriophyids were common in organic and untreated orchards but rare in orchards under conventional spray programs. They were more abundant in summer in untreated orchards (June 2019-2020) and in winter and spring (December 2020, February 2021) in organic orchards. The difference in seasonality might be due to the location of the plots or the varieties. Untreated orchards were located in south Florida (Indian River and St Lucie Counties), while organic orchards were located in central Florida (Lake County). Also, only grapefruits and oranges were present in organic orchards while untreated orchards contained a mix of citrus varieties.

Populations of rust mites were higher on trees at the perimeter than interior of the untreated orchards. The edge effect is shown to influence abundance in other pests such as *D. citri* (Sétamou and Bartels 2015). Edge effects could be related to several factors including pest behavior, environmental factors such as temperatures or sunlight, higher densities of natural enemies in the interior of the plot and surrounding landscapes of the orchards (Haynes and Cronin 2006; Olson and Andow 2008). Indeed, the untreated blocks were surrounded by citrus plantings that may have contributed to the mite infestation in the borders while organic blocks were isolated from other citrus orchards.

Phyllocoptruta oleivora was consistently more abundant (88%) than *A. pelekassi* (12 %) except in one untreated orchard where *A. pelekassi* was dominant. Both species existed in the organic and untreated orchards but *A. pelekassi* was absent from conventional orchards where pesticide applications probably resulted in the suppression of mites and reduced the likelihood of encountering *A. pelekassi*. Resistance to fenbutatin oxide, diflubenzuron, and dicofol has already been reported in *P. oleivora* populations (Childers 1994b; Knapp et al. 1988; Omoto et al. 1994) suggesting that this species is more likely to survive chemical sprays than *A. pelekassi*. Previous studies conducted in commercial citrus orchards in Florida in the 1990's and early 2010 also reported *P. oleivora* in greater abundance (76-77%) compared to *A. pelekassi* (4-23%) (Childers and Achor 1999; Childers et al. 2017). Childers and Anchor (1999) found that out of 120 commercial citrus orchards sampled, 83 had only *P. oleivora,* and 37 had both *P. oleivora* and *A. pelekassi,* but only three had higher frequencies of *A. pelekassi* than *P. oleivora*. Between our and previous studies, *P. oleivora* is still the dominant eriophyid mite species in Florida citrus orchards.

In the canopy, *T. peregrinus*, *Euseius* spp., and *I. quadripilis* were the dominant species, while in the ground cover, *T. peregrinus* and *T. dentilis* were the most prevalent. The same results were found in the survey done in Florida on commercial orchards (Childers and Denmark 2011), suggesting that species assemblage may not be affected by the spray program implemented to control ACP. However, species assemblages differ from the last survey done from 2009 to 2014 by Childers et al. (2022) where *Amblyseius largoensis, E. mesembrinus,* and *E. ovalis* (Evans) were the dominant species in the canopy (i.e., leaves, twigs, and fruit). The authors sampled plots from dooryard, varietal, and some commercial trees with a higher number of varieties including lime, lemon, and tangelo, which were not present in our study. Also, dooryard trees are not under the same cultural practices as commercial orchards and they are mixed with other tree species, while in commercial orchards, stands are conspecific. All these factors could explain the difference in species assemblages compared to our study. *Proprioseiopsis mexicanus* was dominant in the ground cover. It was previously reported as an abundant species in the French West Indies and Alabama citrus orchards (Fadamiro et al. 2009; Kreiter et al. 2018; Mailloux et al. 2010). In the leaf litter, *A. curiosus, P. carolinianus*, *C. floridanus*, and *A. tamatavensis* were the major species. *Chelasieus floridanus* was first described from Florida but was not previously reported as an abundant species. *Amblyseius tamatavensis* is a common species throughout the West Indies (Abo-Shnaf et al. 2016; de Moraes et al. 1999; Mailloux et al. 2010), but its first record in Florida is recent (Döker et al. 2018). According to McMurtry et al. (2013) and their phytoseiid classification, many species of *Propioseiopsis*, *Amblysieus,* and *Chelaseius* are generalist predators subtype IIIe lifestyle from soil or litter habitats. Our results are in agreement with McMurtry's classification since the majority of the *Proprioseiopsis* species sampled were found in the ground cover (such as *P. mexicanus*) or leaf litter (such as *P. carolinianus* and *P. citri*). Moreover, all *Neuseiulus* species identified in our study were encountered in the ground cover except for *N. marinellus* which was also sampled from leaf litter (Demard et al. 2021; Döker et al. 2023). This confirms that *Neoseiulus* are generalist predators inhabiting soil and litter and preferring confined spaces on monocotyledons and dicotyledons plants (McMurtry and Croft 1997; McMurtry et al. 2013).

Phytoseiid populations significantly increased in the canopy from December to April in orchards under all management programs. Similar population dynamics were observed previously in Florida and Alabama citrus orchards (Fadamiro et al. 2009; Muma 1970; Villanueva and Childers 2004). The peak of phytoseiid populations coincides with citrus blooming and pollen production (Villanueva and Childers 2004). In 2020-2021, the increase of phytoseiids was only noticed in conventional orchards. Cold weather in central Florida may have delayed the blooming period and consequently, the increase in phytoseiids in organic orchards (Supplementary Material, Fig. S1 and S2). A larger peak of phytoseiids coinciding with a high abundance of *T. peregrinus* was observed in conventional orchards compared to organic and untreated from December to February of both years. The absence of chemical sprays during this period due to the pre-harvest interval may have favored the reproduction and survivorship of *T. peregrinus* (Supplementary Material, Table S1). Peaks of *T. peregrinus* between February and April were reported in the literature by Childers and Denmark (2011), Fadamiro et al. (2009), and Villanueva and Childers (2004). Furthermore, high densities of *I. quadripilis* and *Euseius* spp. observed in April, in organic orchards, were also reported by Childers and Denmark (2011). Both species are categorized as pollen-feeding generalist predators Type IV by McMurtry et al. (2013) which supports our hypothesis that increase in phytoseiid populations is correlated with pollen availability. In the ground cover, untreated and organic orchards showed similar seasonal abundance, with an increase in *T. peregrinus* populations in June 2020. In conventional orchards, high populations of *T. peregrinus* and *T. dentilis* were noticed in April 2019 and February 2021, respectively. Childers and Denmark (2011) reported similar species patterns with higher populations of *T. dentilis* in February and July as well as an increase of *T. peregrinus* in March-April and July-September. In leaf litter, densities of *A. curiosus* and *N. gracilis* peaked in December and February 2019, respectively, in organic orchards. A similar trend is observed in untreated orchards with *A. curiosus* densities increasing in February 2019. Nevertheless, during 2020-2021, *A. curiosus* was absent from the leaf litter samples except in October and December resulting in low phytoseiids densities for this season.

Typhlodromalus peregrinus was dominant in the tree canopy and the ground cover of conventional orchards, but it was only dominant in the ground cover of organic orchards. *Euseius mesembrinus* could be a better competitor than *T. peregrinus* in the absence of agrochemical sprays, explaining its dominance in the canopy of organic orchards. Yet, *T. peregrinus* might be more tolerant to pesticide applications than *E. mesembrinus*, leading to high abundance in the canopy of conventional orchards compared to orchards under other management schemes.

Even though there were no significant differences, species richness and Shannon indices in the canopy trended higher in organic and untreated orchards compared to conventional. Species in organic and untreated orchards seem to be more evenly distributed than in conventional orchards where *T. peregrinus* represents the majority of the phytoseiids sampled. In the ground cover, species richness was significantly lower in organic orchards. These results are unexpected knowing that in conventional and untreated orchards herbicides were applied (Supplementary Material, Tables S1 and S3). Indeed, the toxicity of herbicides such as glyphosate or paraquat dichloride to phytoseiids was reported by Schmidt-Jeffris and Cutulle (2019), and Mailloux et al.

(2010) showed that applications reduced mite densities and diversity. Cowpea, *Vigna unguiculata* (L.) Walp. was planted as a cover crop in one of the organic orchards sampled. But once it was destroyed, weed populations were not high, in part due to their low survival in sandy soils. The use of cowpea as a cover crop and less weed coverage may be a factor explaining low phytoseiid diversity in the organic orchard. In the leaf litter, Simpson's index and evenness were significantly higher in conventional compared to organic and untreated orchards, suggesting that species were more evenly distributed in conventional orchards. Trees in untreated and organic orchards were usually more severely affected by HLB and other pests since no conventional pesticides were sprayed. As well, trees in untreated orchards were younger and thus smaller, which reduced the amount of leaf litter. These factors could have reduced the quantity and quality of leaf litter present in organic and untreated orchards, affecting phytoseiid populations in this habitat.

The biology of *E. mesembrinus*, *T. peregrinus*, and *I. quadripilis*, the most abundant and frequently encountered phytoseiids, have been studied when they were fed on *P. citri* and *P. oleivora* (Abou-Setta 1988; Abou-Setta and Childers 1987; Fouly et al. 1995; Muma 1967; Peña 1992; Villanueva 2002). Although populations of these three phytoseiids can reach important densities in spring (Childers and Denmark 2011; Demard 2022), none of them seem to provide significant control of *P. citri* and *P. oleivora* in the fields. Other frequently observed species such as *A. tamatavensis*, *T. dentilis,* or *P. mexicanus* could be potential predators for further investigation. *Amblyseius tamatavensis* is a dominant species found in CUPS (Demard 2022) and was observed feeding on *P. citri*. This species was also found in association with *Polyphagotarsonemus latus* (Banks), but no studies have been performed to evaluate its potential on eriophyid or tetranychid mites. Farfan et al. (2021b) found that *P. mexicanus* is a generalist phytoseiid that feeds on various plant pollens but is unlikely to provide an effective control of *Tetranychus urticae* Koch. Moreover, *P. mexicanus* can develop and reproduce on *Tarsonemus bilobatus* Suski, indicating that this species may be a good candidate to control tarsonemid mites of economic importance, such as *P. latus* (Farfan et al. 2021a). The biology and feeding habits of *T. dentilis* have not been studied yet. Childers et al. (2022) recommended *A. largoensis* and *E. ovalis* to suppress mite pests in CUPS. We did not find *E. ovalis* in our samples, but *A. largoensis* was found at low abundances. *Amblyseius largoensis* could also be an effective predator since it feeds on different mite families (Tetranychidae, Eriophyidae, Tenuipalpidae) and it is present throughout the year on Florida coconut trees (Carrillo et al. 2010). Further work is needed to estimate the effectiveness of these species in relation to chemical control and environmental conditions.

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Declarations

Ethical Approval

All authors consent to publish this manuscript.

Competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Availability of data and materials

All data is made available in the manuscript and supplementary materials.

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