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Phytoseiid mite assemblages; Dialeurodes citri and Paraleyroides minei (Hemiptera: leyrodidae) infestations in persimmon orchards under different soil managements in Valencia, Spain

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Phytoseiid Mite Assemblages, *Dialeurodes citri* and *Paraleyroides minei* (Hemiptera: Aleyrodidae) Infestations in Persimmon Orchards under Different Soil Managements in Valencia, Spain

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1. INTRODUCTION

Persimmon (*Diospyros kaki* L.F.), also known as Japanese persimmon or kaki, originates from China and is one of the most important fruit crops in East Asia (Yonemori et al., 2008). It is a perennial deciduous tree that belongs to the family *Ebenaceae.* Its white creamy cryptic flowers transform into green or light green buttons once matured (Britannica, 2021) (Fig. 1a). The simple broad leaves are edible and can be consumed raw or processed (Fig. 1b). The fresh leaves can be cooked and used as a wrap while processed and dried leaves are used as tea for traditional medicine (Xie et al., 2015). The round fleshy orange parthenocarpic fruit is usually consumed as table fruit but can also be processed as sweets or jams (Medha et al., 2019) (Fig. 1c). The nutritious fruit is known as a potential source of beneficial phytochemicals such as antioxidants that are good for human health (Butt et al., 2015; Esteban-Muñoz, 2020). In fact, the name *kaki* in japanese translates to tannin, a phytochemical present in fruits.

Over 500 species of persimmon exist worldwide that are traditionally cultivated in warm sub-tropical climates such as China, Korea, Japan, Brazil, Turkey, Italy (Itamura et al., 2005; Yokozawa et al., 2007; Britannica, 2021). However, this crop has been introduced in other climate regions, such as tropical climates with high altitudes and frequent rainfall but with more or less similar growing conditions (Yuniastuti et al., 2021).

In the Mediterranean basin, persimmon is considered a relatively recent crop dating its introduction to the 19th century in Europe (Perucho, 2015). In Spain, and specifically in the region of Valencia, it used to be a minor crop mostly used for household consumption. The amount of production and area planted nevertheless increased tremendously during the last 25-30 years, making it one of the major crops in the Valencian Community (Tena et al., 2015; García-Martínez et al., 2018). The total land area planted in 2019 was about 16,000 ha, producing nearly 431,000 tons of fruit (GVA, 2019; INE, 2019). The apparent increase is primarily explained by the commercialization of a new cultivar “Rojo Brillante” and the development of an industrial postharvest treatment that eliminates its astringency before ripening. This treatment has permitted fruit transport and storage for more extended periods of time (Arnal and Del Rio, 2003; García-Martínez et al. 2018).

Figure 1. Persimmon *Diospyrus kaki*: (a) flowers, (b) leaves, (c) fruit; and (d) *Festuca arundinacea*

In Spain, the persimmon growing cycle starts in March-April when new buds are formed after dormancy in winter. Ten phenological events were described by García-Carbonell et al. (2002), starting from bud formation to senescence. Leaves mature, and flower buttons appear in the early fruiting stage (May-July). Fruit size continues to increase until September (Intrigliolo et al., 2011). Some fruits start to ripen in early October, turning green to bright orange. Trees are applied with pre-harvest a combination of plant growth regulators. Paclobutrazol (PBZ) is used to advance the ripening while gibberellic acid (GA3) is sprayed to delay (Martínes-Las Heras et al., 2016). In this manner, the farmers can manipulate their harvest season until January, avoiding the peak of the season or when the prices drop (FWS, 2022). Fruit ripening depends on the target market. One is commercialized as a “Classic” tree-ripened, soft-fleshed, deep red-orange color, but short shelf-life. The other is “Persimmon™,” which is picked before full ripening, firm flesh fruit, and a longer shelf-life after CO2 treatment (Llacér and Badenes, 2009). As persimmons are deciduous trees, the leaves are senesced by late autumn to the start of winter, usually by November-December, while the fruit is still attached to the tree. From January to March, the tree is completely defoliated.

With the marked increase in production, monocultural persimmon landscapes have become common in the most important growing areas of the country and concomitantly, several pests such as mites, thrips, lepidopterans and different hemipteran families have been associated more frequently with persimmons (García-Martinez et al., 2018). Whiteflies (Hemiptera: Aleyrodidae) together with mealybugs (Hemiptera: Pseudococcidae) are at the present the two key pest groups in persimmons of Spain (García-Martinez et al., 2019). A benchmark survey of García-Martinez et al. (2018) revealed that whiteflies infestations were mostly due to two species, the citrus whitefly *Dialeurodes citri* (Ashmead) and the nesting whitefly *Paraleyrodes minei* laccarino. Whiteflies feed primarily by sucking phloem content from leaves, leaving little damage unless they reach high populations on the plant. However, their indirect damage if often much more considerable: honeydew secreted by the nymphal instars favors the growth of sooty mold, consequently reducing the marketability of fruits and the photosynthetic ability of leaves.

Current pest management practices in persimmons primarily rely on the spraying of synthetic pesticides. The consequences of this management approach are well documented. The overuse of these products could have long-term effects on human health, the environment, and the naturally occurring biological control processes of agroecosystems (Monzo and Stansly, 2020).

In recent studies in persimmon orchards, important complexes of natural enemies have been found that could contribute to the regulation of the crop pests (García-Martínez 2019). One of the most abundant natural enemies found in this crop were phytoseiid mites (García-Martínez et al. 2016). These predators belong to the family Phytoseiidae consisting of about 2,500 described species worldwide and have a wide range of dietary habits, including pollen, fungi, nematodes, mites, and other small arthropods. Its predatory behavior has attracted particular attention on the biological control of agricultural pests (Denmark, 2008). Phytoseiids are important natural enemies of whiteflies in vegetables and could therefore play an important role on the regulation of these phytophagous in persimmon orchards. Previous studies in Valencia region found 5 species of phytoseiid mites associated with this crop: *Euseius stipulatus* (Athias-Henriot), *Typhlodromus phialatus* Athias-Henriot, *Amblyseius andersoni* (Chant), and *Paraseiulus talbii* (Athias-Henriot) (García-Martínez et al. 2019) and later *Amblyseius swirskii* Athias-Henriot (Hernández-De la Fuente, 2018). However, their role as biological control agents of persimmon pests remain unknown. Phytoseiids are sensitive to intensive pesticide programs (Monzo and Stansly, 2016) and the current insecticide pest management practices in this crop may impact their abundance and hamper their biological control activity. Pesticides affect phytoseiids in different ways depending on the active ingredient and the phytoseiid species (Fiedler and Sonsnowska, 2014; Salman et al., 2015). Consequently, the species composition as well as their relative abundance in phytoseiid assemblages is also expected to be affected by insecticide usage (Monzo and Stansly, 2016).

In search for more environmentally friendly options, conservation biological control (CBC) strategies seek to minimize the adverse effect of agricultural practices on non-target and beneficial organisms, such as Phytoseiid mites, and to enhance their activity and role as biological control agents (Begg et al., 2017). Habitat manipulation practices aim at increasing the complexity of agroecosystems to favor the presence of natural enemies by providing them a wider range of habitat and food resources (Begg et al., 2017). The use of cover crops in the alleyways of perennial crops as a CBC strategy has been widely studied in some crops but no research has been developed in persimmon. In citrus, monospecific cover crops sown with *Poaceae* enhanced phytoseiid mites biological control activity in the tree canopy thanks to bottom-up regulation mechanisms , when agroecological habitat manipulation at lower trophic level (the plant) controls the higher trophic organisms (pest) (Aguilar-Fenollosa, 2011).

Given the fact that persimmon orchards shelter diverse and abundant Phytoseiid assemblages that could contribute to more effective integrated pest management strategies, the objective of the present research was to evaluate how sown cover crops of *Festuca arundinacea* Schreb (Poaceae) (Fig. 1d) may affect phytoseiid assemblages and whitefly incidence in persimmon orchards.

2. MATERIALS AND METHODS

2.1 Study sites

Eight commercial persimmon orchards of approximately 0.5 ha each, located in two towns of the Valencia region; L'Alcudia, and Alginet, were included in this study (Tab. 1). The longest distance between groves was 13 km, the altitude of the study area ranged between 20 and 40 meters, and consequently, all study sites shared similar environmental conditions (Fig. 2). The climate in the study area is characterized as Mediterranean type, with warm and temperate temperatures, experiencing more precipitation in winter months than in summer (499mm/year). All groves were in full commercial production. Hence, conventional pest management was inevitable. Pesticide applications were made between May 1st and August 30th with biweekly insecticide applications against mealybugs following a rotation of Movento® (spirotetramat), Closer® (Sulfoxaflor), Epik (Acetamiprid) and Karate Zeon (Lambda cihalotrin). All orchards received the same insecticide application. The soil in the alleyways was differentially managed between groves. Four groves, located in L'Alcudia, were kept with plant-free alleyways by periodical applications of Glyphosate (Fig. 3a). In contrast, four orchards, located in Alginet, maintained a monospecific cover crop of the grass *Festuca arundiancea* Shreb that was sown in October 2019 (Fig. 3b). The cover crop is mowed four times per season in February, April, June, and September.

2.2 Sampling of predatory mites and whiteflies

Sampling in the eight selected orchards was done approximately every other week from April to September 2021. In each sampling date, trees were sampled by randomly collecting a shoot of approximately 10 leaves of the canopy of 10 randomly selected trees per study site. The samples were subsequently taken to the laboratory and stored at a cold temperature (approx. 5°C) until inspection. The leaves were carefully examined under a stereomicroscope and the number of whiteflies, as well as its developmental stage (egg, nymph or adult) per leaf was noted. Simultaneously, all the mites found were counted, extracted and preserved temporarily in Eppendorf tubes containing 70% alcohol for further taxonomic determination.

In the groves sown with *F. arundinacea*, three sampling points were selected per the orchard following a diagonal line, and in each point, a composite sample of about 150 g of leaf vegetation of the cover crop was mowed and stored in plastic zip-bags. Samples were taken to the laboratory and placed in Berlese-Tullgren funnels during 48 h for mite extraction. At the bottom of each funnel, a collection tube was placed containing 60% ethanol, 10% glycerin, 0.3% detergent, 0.1% sodium benzoate, and water to make 100% of the mixture (Ferragut et al, 2010).

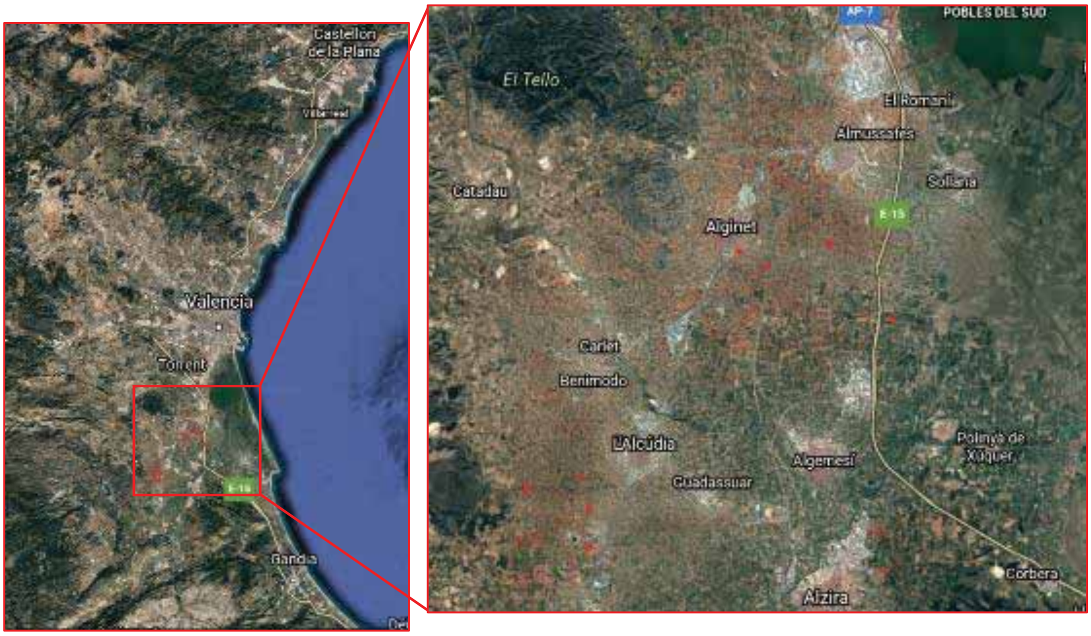


Figure 2. Location of the eight sampling sites of the study marked in red dots.

Figure 3. The sample orchards with (a) bare soil and (b) with *F. arundinacea*

Table 1. Characteristics of the eight commercial persimmon orchards selected for the study.

Orchard	Name	Town	Geographic Coordinates	Presence of cover crop *F. arundinacea*	Size (ha)
1	La Carriona	L'Alcudia	39°11'04.2"N 0°32'58.4"W	-	0.601
2	El Minat	L'Alcudia	39°11'16.8"N 0°31'49.5"W	-	0.191
3	Rincon Monzon	L'Alcudia	39°10'42.1"N 0°31'32.2"W	-	0.244
4	Monleon	L'Alcudia	39°09'59.0"N 0°31'32.3"W	-	0.466
5	Mitgera	Alginet	39°15'24"N 0°28'01"W	+	0.391
6	Moncara	Alginet	39°15'32"N 0°25'52"W	+	0.639
7	Vall d'Hebron	Alginet	39°15'08"N 0°27'20"W	+	0.303
8	Vintena	Alginet	39°14'08"N 0°24'26"W	+	0.843

2.3 Taxonomic identification of Phytoseiid mites

All the predatory mites of the family Phytoseiidae collected both from persimmon leaves and *F. arundiancea* were counted. Phytoseiid mites appear bigger, slightly transparent or sometimes brown-pigmented, with glossy cuticle, and highly agile compared to other saprophytic or phytophagous mites on the leaves (Fig. 4). These characteristics were certified before extracting them and preserve in 70% ethyl alcohol. To aid in the identification, the body extracted mites were digested and cleared using Nesbitt's solution for 24 hours at 40-50°C and later mounted in Hoyer's medium. The important taxonomic features (body shape, number, and location of setae, shape, and texture of dorsal shield, ventral plates, and spermatheca) were examined under phase-contrast microscopy. The taxonomic key of Ferragut et al. (2010) was used for identification to genus, or if possible, to the species level. Adult females were selected for species identification as it is more reliable than using males or immatures. However, immatures and males were still considered for counting the total number of Phytoseiids.

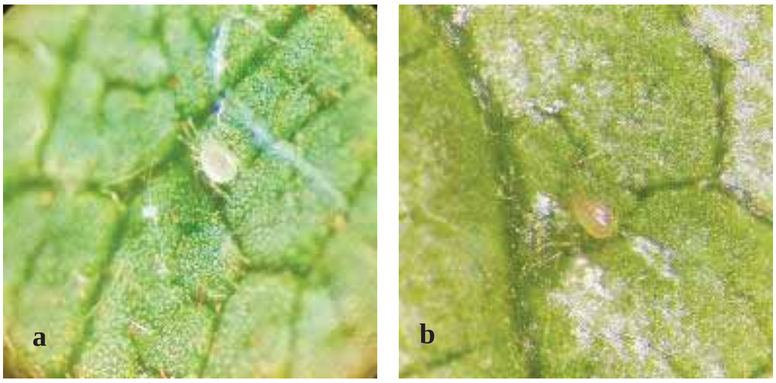


Figure 4. The appearance of transparent (a) and slightly pigmented (b) phytoseiid mites viewed under a stereomicroscope

2.4 Statistical analyses

Analysis of variance, of the total number of whitefly adults, nymphs, eggs and total phytoseiid mites per persimmon leaf was done following the Nested design (Factor A: soil management, Factor B: location) using the program *R* studio ver 3.6.1 (R Development Core Team, 2019) and its packages “agricolae”, “lsmeans”, “dplyr”, and “ggplot”.

3. RESULTS

3.1 Seasonal abundance of whiteflies and phytoseiid mites

Table 2 shows the average counts of whiteflies and phytoseiids per leaf throughout the sampling period for the bare soil orchards and orchards with cover crops. Two whitefly species were found throughout the study, the citrus whitefly *D. citri* and the nesting whitefly *P. minei*. In terms of developmental stages, more eggs than adult individuals per leaf were recorded in both species. Generally, more *D. citri* were found in persimmon leaves than *P. minei* in all developmental stages. Nymphs and eggs of *D. citri* were recorded more than twice the number of *P. minei*, while adult counts did not differ a lot.

The cover crop had a profound effect on reducing the nymphs and eggs of *D. citri* per leaf ($P<0.0001$). Groves with bare soil had about 3.9 and 2.7 times more nymphs and eggs per leaf, than the covered soil. However, a higher number of adults was recorded in persimmon orchards with cover crop. Meanwhile, the effect of cover crop is also significant in *P. minei*. Fewer number of *P. minei* was noted in orchards with *F. arundinacea* than those that were uncovered. This is true in all developmental stages with great effect observed on eggs, about 11-fold less.

The number of Phytoseiid mites per leaf was significantly higher in orchards with cover crops ($P=0.0324$). It is about 1.4 higher than in persimmon groves left with bare soil. In both species of whiteflies, the average egg count outnumbered the Phytoseiids. This was observed in both orchards except for *P. minei* in ground-covered soils.

Table 2. Mean ± standard error of the density of whiteflies (adults, nymphs, eggs) and phytoseiid mites per leaf collected in four orchards with and without cover crop across the sampling period from April 2021 to September 2021. LSD test was used to compare the effects of the type of soil management (with *F. arundinacea* and bare soil) Results are averaged over the levels of the location.

Parameter	Bare Soil	Cover Crop	F	*Df*	*P*
D. citri					
Adult	0.17 ± 0.47	0.43 ± 0.47	15.7	1, 72	0.0002
Nymph	8.88 ± 0.63	2.26 ± 0.63	55.0	1, 72	<0.0001
Egg	22.9 ± 1.46	8.40 ± 1.46	49.5	1, 72	<0.0001
P. minei					
Adult	0.15 ± 0.03	0.00 ± 0.03	16.9	1, 72	0.0001
Nymph	1.23 ± 0.87	0.04 ± 0.87	28.4	1, 72	<0.0001
Egg	9.79 ± 0.80	0.32 ± 0.80	68.8	1, 72	<0.0001
Phytoseiids	4.86 ± 0.45	6.80 ± 0.45	4.76	1, 72	0.0324

Seasonal trends of all developmental stages of *D. citri* were more or less similar between groves with and without cover crop (Fig. 5). In persimmon orchards with bare soil, two peaks were observed in the population dynamics of *D. citri* adults, one at the beginning of the growing season (end of April) and the second one in mid-July. Then the adult counts remained low for the rest of the season, about less than 1 individual per tree. Nymphs and eggs appeared two weeks after the first population peak of adults. The second population surge happened in July and remained lower at the end of the sampling period. Meanwhile, egg numbers re-increased about 23 individuals per tree in mid-August, but not as high as the 1st and 2nd peak.

Orchards with cover crop recorded fewer *D. citri* in all developmental stages except the adults. The number of *D. citri* adults found in the groves with *F. arundinacea* at the beginning of the growing season was approximately six times higher than in those with conventional soil management. However, population trends did not differ much from orchards with bare soil throughout the sampling period, apart from the 2 peaks. Nymphs, on the other hand, had the highest peak in June, 2 weeks later than the 1st peak of nymphs found in orchards with bare soil. It continued to decline and remained below 5 individuals per tree until September. Oviposited eggs per tree were also fewer in the 1st and 2nd peaks and did not resurge in August, unlike the ones in bare soil. Interestingly, egg counts dropped equally in both types of groves, with about 13 and 10 eggs in the 1st and 2nd peaks, respectively.

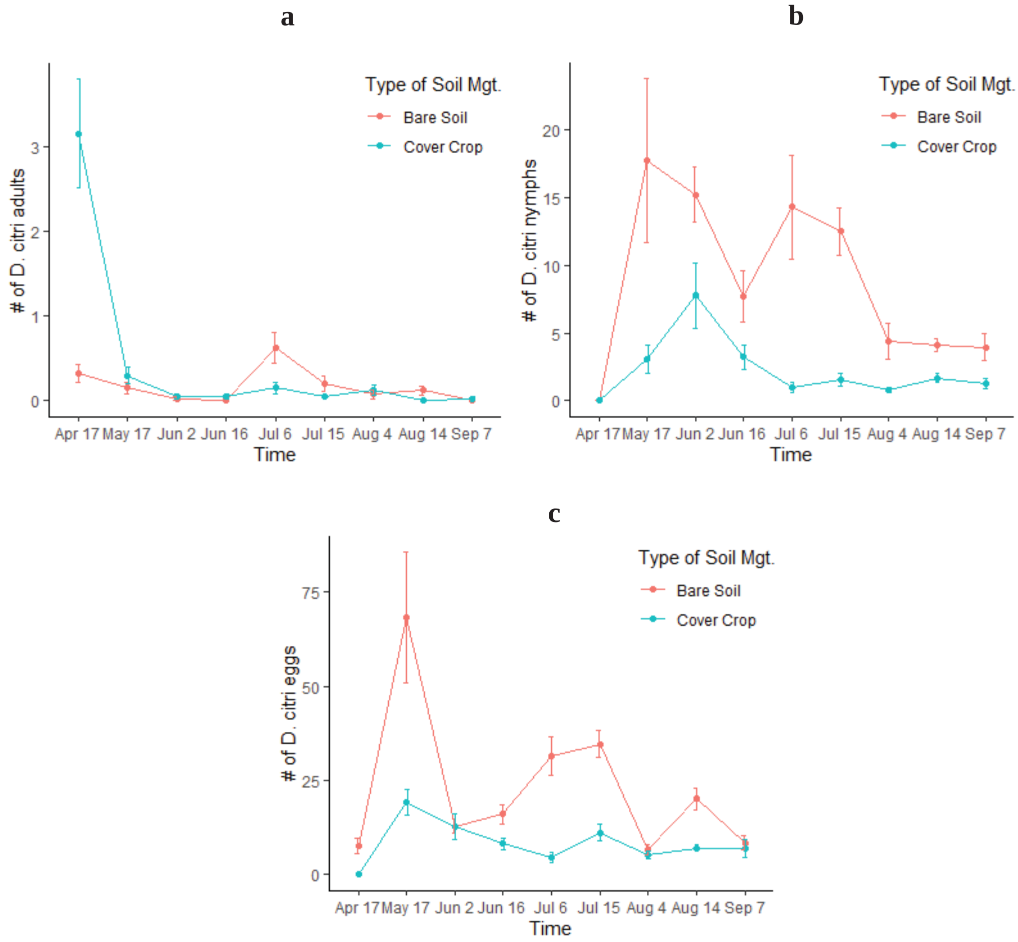


Figure 5. Total number of *D. citri* (a) adults, (b) eggs, (c) nymphs collected per persimmon tree (n=10) in orchards with *F. arundinacea* and bare soil.

Another whiteflies species, *Paraleyrodes minei*, was observed later in the season (June) than *D. citri*, which started to appear in April (Fig. 6). All developmental stages of this nesting whitefly appeared at the first sampling date of July. In soils without ground cover, the population increased until the last collection period. This was true for nymphs and eggs, but not with adults which had a fluctuating population. It declined in August, regained its population after 2 weeks, and finally declined at the last sampling. On the other hand, soils covered with *F. arundinacea* had very few or almost absent *P. minei* across all life stages. Few sampling periods overlapped with bare ground, one in August for adults, and one in July for nymphs and eggs.

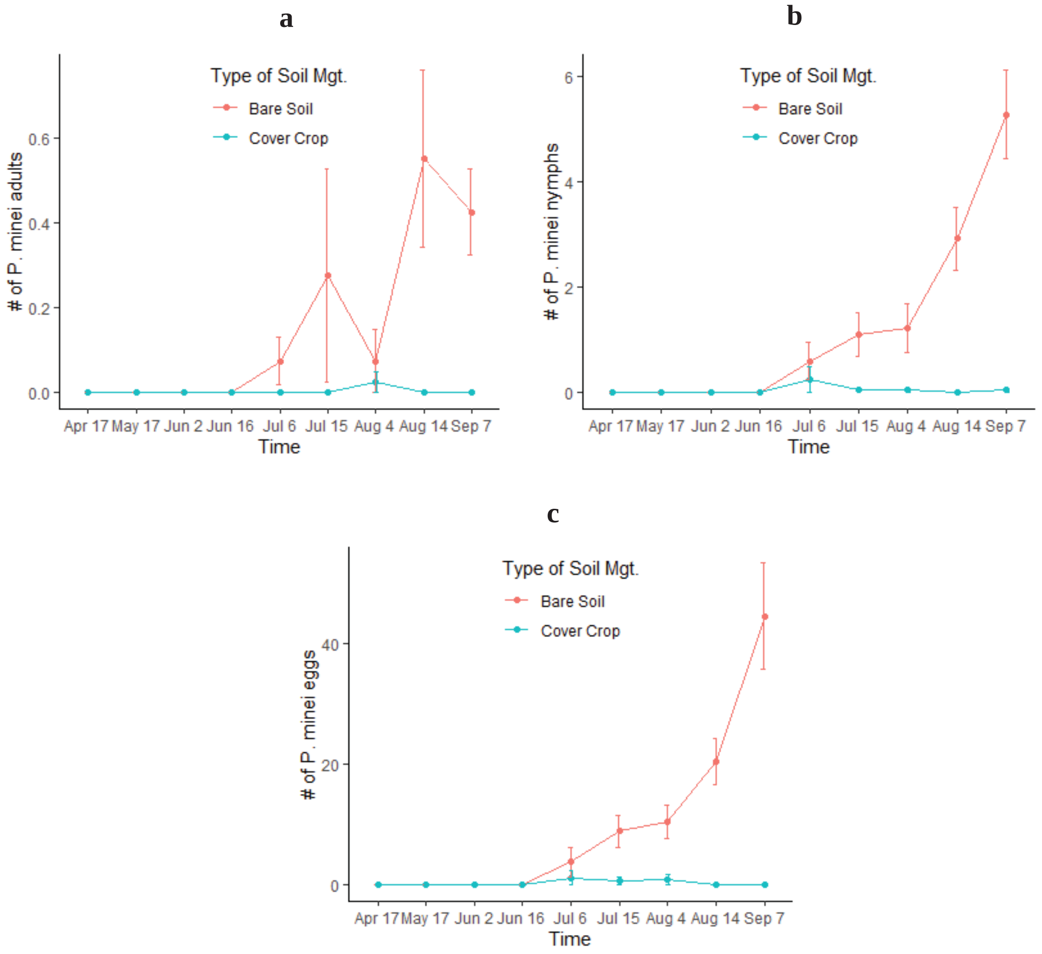


Figure 6. Total number of nesting whitefly *P. minei* (a) adults, (b) eggs, (c) nymphs collected per persimmon tree (n=10) in orchards with *F. arundinacea* and bare soil.

Phytoseiid mites' seasonal trend is shown in Figure 7. In the first two sampling dates, populations were the same regardless of the type of management. This was also true at the last two samplings. Notable differences were recorded from June to mid-August, when more phytoseiids were found in groves with a cover crop, except on 6 July. It showed 2 peaks in both types of soil management. The 1st increase was observed in June and the 2nd in September.

Additionally, ground-covered groves had more Phytoseiids in the 1st peak compared to the bare soil but had the same counts in the 2nd one. It is also interesting to note that the two increments occurred just after the *D. citri* peaks that happened in 17 May and 15 July (Fig. 5). Abundance of these predatory mites was especially higher in the groves with *F. arundinacea* than in the groves with bare soil during the first peak in mid-June.

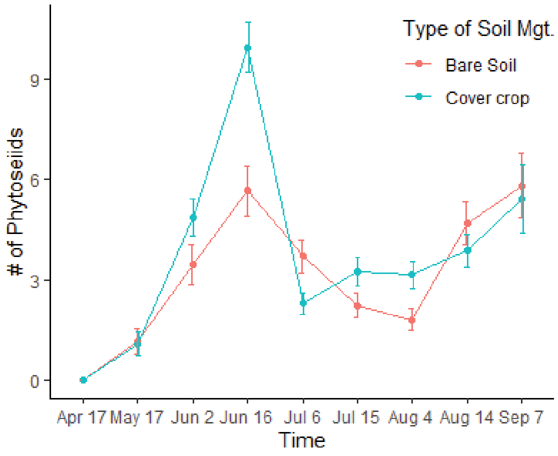


Figure 7. Population dynamics of Phytoseiid mites collected per tree (n=10) in both type of soil managements across sampling dates.

3.2 Species composition and relative abundance of Phytoseiid assemblages in persimmon trees

A total of 1,331 Phytoseiid adult females belonging to 6 different species (Anex Fig. 1-6) were collected in the canopy samples of the eight persimmon orchards between April 2021 and September 2021. Four species belonged to subfamily *Amblyseiinae: Euseius stipulatus* Athias-Henriot, *Proprioseiopsis messor* Wainstein, *Amblyseius andersoni* Chant, and *Neoseiulus barkeri* Hughes. The other two species, *Typhlodromus phialatus* Athias-Henriot, and *Paraseiulus talbii* Athias-Henriot, belonged to subfamily *Typhlodrominae*. The groves with *F. arundinacea* cover crop had more predatory mites on the leaves than the groves with bare soil, accounting for 749 and 582 individuals, respectively (Table 3). Both types of orchards had the same species richness of 5 species, but differed in the species composition. Four species, *E.*

stipulatus, T. phialatus, P. talbii, and *N. barkeri* were common in both orchards. On the other hand, no *P. messor* was found in any orchards with bare soil, while in the orchards with cover crop, the species *A. andersoni* was absent.

Table 3. Abundance of adult Phytoseiid species on persimmon leaves in orchards with and without a ground cover of *F. arundinacea* over all sampling dates

Species	Orchards with bare soil 1	2	3	4	Total No.	Orchards with cover crop 5	6	7	8	Total No.
Euseius stipulatus	82	147	120	80	429	169	166	122	154	611
Typhlodromus phialatus	30	34	43	41	148	23	53	32	26	134
Paraseiulus talbii	0	2	0	0	2	1	0	0	0	1
Amblyseius andersoni	0	0	2	0	2	0	0	0	0	0
Proprioseiopsis messor	0	0	0	0	0	1	1	0	0	2
Neoseiulus barkeri	0	1	0	0	1	1	0	0	0	1
Total	112	184	165	121	582	195	220	154	180	749

The relative abundance of the Phytoseiid species in the different groves is shown in Figure 8. In the groves with cover crops *E. stipulatus* amounted to 81.7% of the specimens, *T. phialatus* to 17.8%, *P. messor* to 0.2%, and *P. talbii* and *N. barkeri* to 0.1%. The two most dominant species were present in all 4 sample farms, while the rest only appeared in 1 or 2.

In the orchards without ground cover, the collected phytoseiids consisted of 73% *E. stipulatus*, 26.3% *T. phialatus*, 0.3% *P. talbii*, 0.1% *A. andersoni*, and 0.1% *N. barkeri* (Figure 8). Thus, the two species, *E. stipulatus* and *T. phialatus* dominated in both types of orchards, regardless of the soil management, while other species are almost insignificant in terms of numbers.

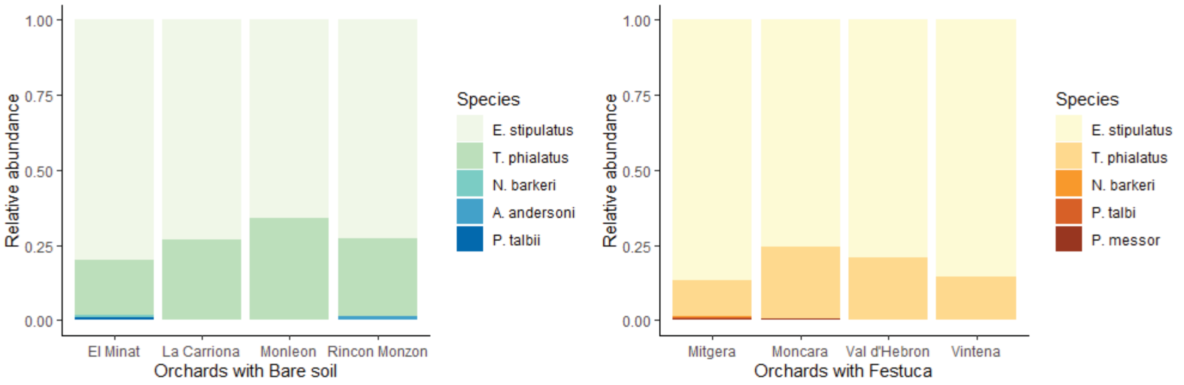


Figure 8. Relative abundance of phytoseiid species collected from April 2021 to September 2021; (a) orchards with *F. arundinacea*, (b) orchards with bare soil

3.3 Species composition and relative abundance of Phytoseiid assemblages in *Festuca arundinacea*

Direct sampling of the cover crop revealed a different phytoseiid species composition than on persimmon leaves. A total of 6 species, *Proprioseiopsis messor*, *Neoseiulus barkeri*, *N. californicus* McGregor (Annex Fig 7), *E. stipulatus, T. phialatus,* and *Amblyseius obtusus* Koch (Annex Fig. 8) were found on the cover crop from the four sampled orchards (Figure 9). The species collected and their relative abundance varied among orchards. In general, *P. messor* and *N. barkeri* were predominant in all orchards, ranging from 18.2–81.3% and from 6.2–68.2% of the total phytoseiids, respectively. The dominant species in the canopy of persimmon trees, *E. stipulatus* and *T. phialatus*, were uncommon and almost absent on the ground cover. On the contrary, the most abundant species in cover crop, *P. messor*, was rare in the canopy.

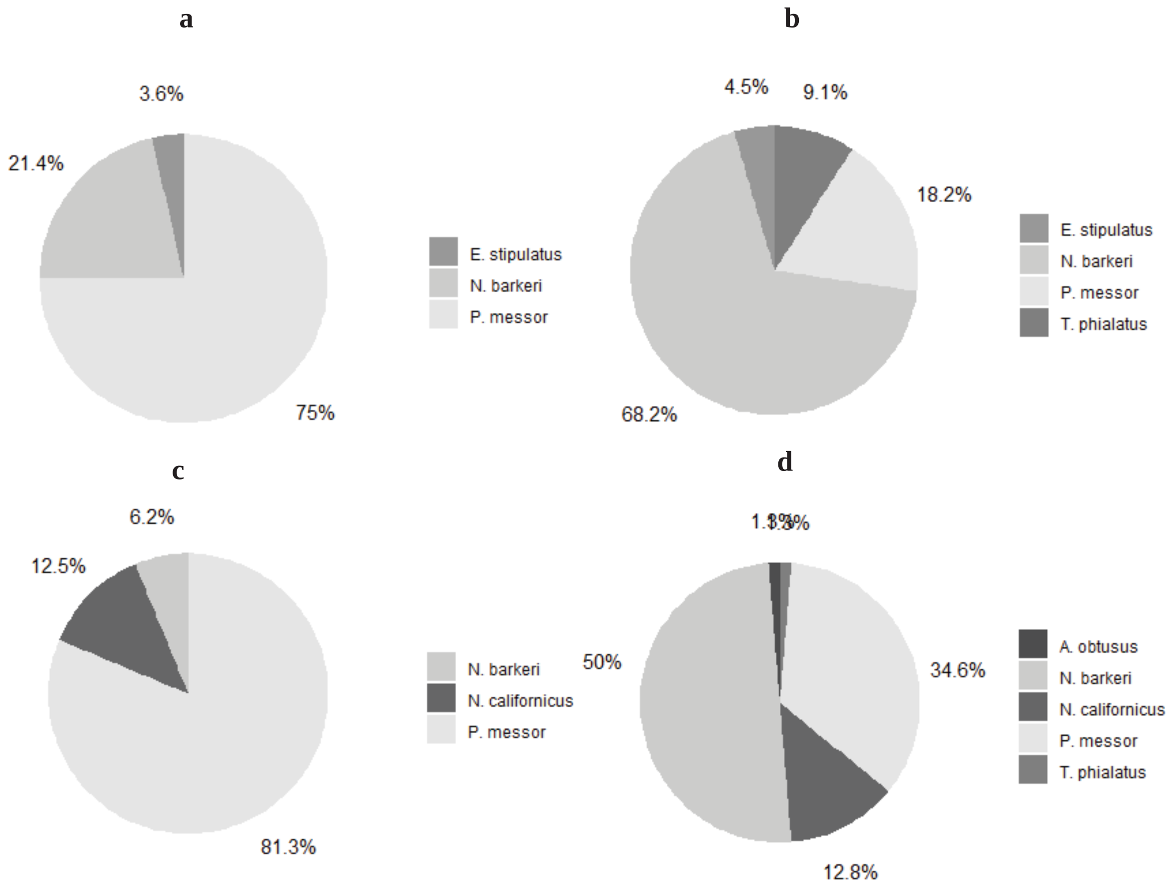


Figure 9. Relative abundance of Phytoseiids collected from *F. arundinacea* used as cover crop in four persimmon orchards, (a) Mitgera, (b) Moncara, (c) Val d'Hebron, (d) Vintena.

4. DISCUSSION

As the land area grown with persimmons increases, pest problems such as whiteflies have emerged. The presence of these harmful Hemipteran pests indirectly affect the market value of the product because of sooty mold growth. The non-judicious application of synthetic pesticides to kill this pest poses a threat to humans and the environment. In this research, I studied the potential of sown cover crops with *F. arundinacea* in augmenting the populations of Phytoseiid mites, a vital predatory group for conservation biological control, as an alternative for synthetic insecticide application.

The present 6-month study on the population dynamics of *D. citri* and phytoseiid mites, despite the short period, suggests that the sown cover crop, *F. arundinacea*, may help to reduce the nymph and egg abundance of *D. citri*, but not adults. The possible explanation, why the adults were higher is that they are highly mobile, unlike nymphs and eggs, which remain on the leaves. Plots with ground cover had a higher number of adults at the beginning of the sampling. The first arrival of whitefly adults is thought to be from nearby citrus groves in early spring when persimmon leaves are young. The population dynamics of whiteflies showed two population peaks, in May and July, suggesting two generations, regardless of the presence of cover crops. It is known that *D. citri* usually has three annual generations, the first two coinciding with what has been observed in this study and the third one is given around September (Hernández-De la Fuente et al., 2019; Argov et al., 2003; Žanić et al., 2001).

Another species of whitefly, *P. minei*, was also found later in the season. Both types of orchards had no *P. minei* initially but started to have significant differences later in the season. Unlike *D. citri,* only one peak of population was observed for both eggs and immatures but not for adults, suggesting only one generation. In field data observed in California, where the climate is also classified as Mediterranean type, this species has at least four generations throughout the year recorded in citrus (Bellows et al., 1998). The first increase in numbers of all life stages of *P. minei* was observed at the same time as the 2nd generation of *D. citri*, whose abundance is lower than that of its first generation. This could suggest that both species in persimmon might have an interaction during this time of the growing season.

The increase in phytoseiid numbers was evident two weeks after the first population peak of *D. citri*, indicating that the predator may use the immature stages of the whitefly as prey and numerically respond to their densities. The late increase in the numbers of the uncommon species of whitefly, *P. minei,* might be an additional food source for the predatory mites aside from *D. citri,* despite its lower numbers in the 2nd generation. Two species dominated phytoseiid assemblages of persimmons*, E. stipulatus,* and *T. phialatus,* that accounted for about 80% and 20% of our total captures, respectively. The generalist diet and direct plant-feeding ability of *E. stipulatus* could explain its superiority and survival advantage among other species (Cruz-Millares et al., 2021). These dominant species might be the most crucial guild in conservation biological control. The results agree with previous studies on Phytoseiids in persimmon from conventional and non-conventional farming systems. In a three-year investigation, Garcia-Martinez et al., (2019) found four species on the tree leaves, namely *E. stipulatus, T. phialatus, A. andersoni,* and *P. talbii*. The latter two species were less common and were only found in one of the three years of sampling. On the other hand, another study conducted in 2017 (Hernández-De la Fuente, 2018) showed that *Amblyseius swirskii* Athias-Henriot was the most abundant phytoseiid in 3 of the 4 sampled orchards in the region. This predatory mite is an introduced species for biological control purposes in greenhouses, as it is one of the key natural enemies of whiteflies on vegetables (Calvo et al. 2011). In 2017, the species was released on persimmon to keep whitefly populations under control in the same region of L'Alcudia and Alginet, Valencia. Nevertheless, *A. swirskii* was absent in our samplings indicating that the species probably did not survive in the area after the releases. My study found two more species not previously reported in the canopy of persimmons, *P. messor* and *N. barkeri*. In Spain, *P. messor* was reported to be associated with tomato, maize, stone fruits, grapes, and spontaneous vegetation like cover crops (Ferragut et al., 2010). *N. barkeri* is a common generalist predator commercially available for biological control of some agricultural pests (Cintra-Filgueiras et al., 2020).

As the persimmon groves are in full commercial production, the application of insecticides was unavoidable. Spraying events that happened every two weeks may have indirectly affected the population dynamics of mites and whiteflies. However, it does not reflect on the population curve. For example, application on 15 May, might have reduced the *D. citri* adults but not the nymphs and eggs, which increased on the 17 May sampling date. Phytoseiid mites' population also started to grow on 17 May, 2 June, and continued until its highest peak in 16 June, just a few days after insecticide treatment. Hence, we can rule out the direct effect of insecticides. However, studies related to Phytoseiid mites-whiteflies population dynamics, without any application of pesticides could also be another topic but are difficult to conduct because persimmons are mostly grown for commercialization purposes.

The two phytoseiids predominant on the ground cover, *P. messor* and *N. barkeri* were rare on the canopy of persimmons. These species appear to be more associated with low-lying crops close to the ground and could explain why they are almost absent in the tree canopy. Aguillar-Fonollosa et al. (2011) previously reported the presence of these species in *F. arundinacea* grown as cover crops in citrus orchards. Interestingly, the authors also revealed that the most abundant species in the canopy, *E. stipulatus* and *T. phialatus*, were the least in the cover crop. In a study conducted by Tixier (2018), some phytoseiid mite species appear to have preference on crops and non-crop plant. *E. stipulatus*, for example, appears to favor more on herbaceous plants than arboreal plants, while *Typhlodromus pyri* and *Kampimodromus aberrans* prefer the latter. The preference of predatory mite species might explain why some species were dominant in the cover crop but not in persimmon trees.

I hypothesized that there might be a movement of species from the cover crop to the canopy of persimmons when the leaves start to grow in spring or the other way around when persimmons defoliate during autumn. In this way, the presence of cover crop might be beneficial to the phytoseiid mites seeking refuge at harsh times. However, the phytoseiid assemblage in the persimmon canopy differs from ground sampled in early spring to late summer (15 April 2021 to 7 September 2021). The most abundant species in the tree are almost absent on the ground. In contrast, the most abundant species on the soil are rare in the canopy, indicating that the movement of species might not have been possible during the time of the study. Further research can be done in autumn or winter to see whether we can see a similar assemblage of mites in the cover crop and the canopy.

Even though the findings indicate that phytoseiid assemblages differ between persimmon trees and cover crop, the higher abundance of phytoseiid mites in the tree canopy of orchards with cover crop suggests some interaction between both groups. This hypothesis will be tested in further research. Cover crops of *F. arundinacea* could therefore serve as a reservoir of these natural enemies as has already been demonstrated in other crops such as citrus (Aguilar-Fenollosa et al. 2011).

5. SUMMARY

The increasing production and the plantation area of persimmons in Valencia, Spain, has been beneficial in the region's economy. However, with the astounding production increase, new pests are being discovered in this relatively new perennial tree that originated from Asia. One of the most critical pests associated with persimmons are whiteflies. Two species, namely citrus whitefly *Dialeurodes citri* and nesting whitefly *Paraleyrodes minei*, started to feed on persimmons and indirectly caused sooty mold by its honeydew. It was previously known that persimmons harbor a diverse assemblage of phytoseiid mites. Still, little is known if cover crops can be beneficial to increase the populations of the phytoseiid mites, thus increasing its predatory activity.

In this study, I hypothesized that the phytoseiid mite assemblage in persimmon canopy is the same as the assemblage in the cover crop and the presence of the cover crop *Festuca arundinacea* is beneficial to these phytoseiid mites. In return, this could better regulate the population of two whiteflies species, *D. citri* and *P. minei.* Eight persimmon orchards in Valencia were sampled every other week from April 2021 to September 2021. Half of the orchards had a spontaneous cover crop, *F. arundinacea,* while the other half had bare soil between trees. The phytoseiid mites present on the leaves were counted and identified. Adults, nymphs and eggs of the two species of whiteflies were also measured.

Two whitefly species were found, *D. citri* and *P. minei.* The former appeared earlier in the season and had at least two peaks of its population while the latter appeared at mid-season and coincided with the 2nd peak of *D. citri.* The incidence of both species was higher in the groves without the cover crop, suggesting that the presence of cover crop may help reduce the incidence of the whiteflies. On the other hand, the increment of the phytoseiid mites’ population was evident after the population peak of the whiteflies, indicating a predator response to an increase of prey abundance. The results indicate that the presence of Poaceae cover crop may help to reduce the incidence of the two whiteflies species associated to persimmon orchards thanks to the enhanced phytoseiid mite assemblage populations.

The species composition and relative abundance of the female phytoseiid mites in both types of orchards are more or less the same and seem to be unaffected by the presence of the cover crop. Additionally, it was different from the canopy and the cover crop. The most abundant species in the canopy were rare in the ground, and the most abundant ones in the soil were almost absent in the tree. This might suggest the movement of species is not possible from the cover crop to the canopy or the other way around in the harsh conditions. This study should be continued to see whether the trend will continue in autumn and winter.

6. REFERENCES

Aguilar-Fenollosa E, MV Ibáñez-Gual, S Pascual-Ruiz, M Hurtado, JA Jacas (2011). Effect of ground-cover management on spider mites and their phytoseiid natural enemies in clementine mandarin orchards (II): Top-down regulation mechanisms. *Biological Control* 59: 171-179.

Argov Y, Y Rössler, H Voet, D Rosen D (2003). The biology and phenology of the citrus whitefly, *Dialeurodes citri*, on citrus in the Coastal Plain of Israel. *Entomologia Experimentalis et Applicata* 93(1):21 – 27.

Arnal L, MA Del Río (2003). Removing astringency by carbon dioxide and nitrogen-enriched atmospheres in persimmon fruit cv.“Rojo brillante”." *Journal of Food Science* 68(4): 1516-1518.

Begg GS, SM Cook, R Dye, M Ferrante, P Franck, C Lavigne, GL Lövei, A Mansion-Vaquie, JK Pell, S Petit, N Quesada, B Ricci, SD Wratten, ANE Birch (2017). A functional review of conservation biological control. *Crop Protection* 97: 145-158.

Bellows JR, Meisenbacher C, Headrich DH (1998). Field biology of *Paraleyroides minei* (Homoptera: Aleyrodidae). *Population Ecology* 27 (2): 277-281.

Britannica, The Editors of Encyclopedia (2019). "Diospyros". *Encyclopedia Britannica*, 7 Nov. 2019. URL: https://www.britannica.com/plant/Diospyros, accessed on 14 October 2021.

Butt MS, MT Sultan, M Aziz, A Naz, W Ahmed, N Kumar, M Imran (2015). Persimmon (*Diospyrus kaki*) fruit: hidden phytochemicals and health claims. *Experimental and Clinical Journal* 14: 542-561.

Calvo, FJ, K Bolckmans, JE Belda (2011). Control of *Bemisia tabaci* and *Frankliniella occidentalis* in cucumber by *Amblyseius swirskii*. *BioControl* 56(2): 185-192.

Cruz-Miralles J, M Cabedo-López, M Guzzo, V Ibáñez-Gual, V Flors, JA Jaques (2021). Plant-feeding may explain why the generalist predator *Euseius stipulatus* does better on less defended citrus plants but *Tetranychus*-specialists *Neoseiulus californicus* and *Phytoseiulus* *persimilis* do not. *Experimental and Applied Acarology*. 83(2): 167-182. DOI: 10.1007/s10493-020-00588-x.

Denmark HA, (2008). Phytoseiid Mites (Acari: Phytoseiidae). In: Capinera J.L. (eds) Encyclopedia of Entomology. Springer, Dordrecht. DOI: 10.1007/978-1-4020-6359-6_2950.

Esteban-Muñoz A, S Sánchez-Hérnandez, C Samaniego-Sánchez, R Giménez-Martínez, M Olalla-Herrera (2020). Differences in the phenolic profile by UPLC coupled to high resolution mass spectrometry and antioxidant capacity of two *Diospyros kaki* varieties. *Antioxidants* 10(31). DOI: 10.3390/antiox10010031.

Ferragut F, I Perez-Moreno, V Iraola, LA Escudero-Colomar (2010). Acaros depredadores en las plantas cultivadas. Ediciones Agrotécnicas, S.L Madrid. ISBN: 978-84-87480-53-9.

Fiedler Z, D Sosnowska (2014). Side effects of fungicides and insecticides on predatory mites, in laboratory conditions. *Journal of Plant Protection Research* 54(4) DOI: 10.2478/jppr-2014-0052.

FWS-Food and Wines from Spain (2018). “Spanish Persimmons: The sweetest things!” in Food and Wines from Spain website. 22 November 2018. URL: https://www.foodswinesfromspain.com/spanishfoodwine/global/food/features/feature-detail/spanish-persimmons.html#:~:text=Persimmon%20season%20in%20Spain%20is,fruit%20to%20get%20their%20fill.

Cintra-Filgueiras RM, JA Mendes, EP De Sousa Neto, NV Monteiro, JW Da Silva Melo (2020). *Neoseiulus barkeri* Hughes (Acari: Phytoseiidae) as a potential control agent for *Raoiella indica* Hirst (Acari: Tenuipalpidae). *Systematic and Applied Acarology*. 25(4): 593-606. DOI: 10.11158/saa.25.4.1

García-Carbonell S, B Yagüe, H Bleiholder, H Hack, U Meier, M Agustí (2002). Phenological growth stages of the persimmon tree (Diospyrus kaki). *Annals of Applied* Biology 141: 73-76.

García-Martínez O, F Beitia, J Baixeras, L Torres, A Urbaneja, M Pérez-Hedo (2016). Barrenetas presentes en el cultivo del caqui en la Comunidad Valenciana. *Agrícola Vergel* 397:318–322.

García-Martínez O, A Urbaneja, F Beitia, M Pérez-Hedo (2018). Basis for implementing an integrated pest management program (IPM) in persimmon. *Acta Horticulturae* 1195: 141-146.

García-Martínez O, (2019a). Bases para una gestión integrada de plagas en caqui en la Comunidad Valenciana (Doctoral dissertation, Universitat d'Alacant-Universidad de Alicante), 136pp.

García-Martínez O, M Pérez-Hedo, A Urbaneja, F Beitia (2019b). Principales fitófagos en el cultivo de caqui de la Comunidad Valenciana: evolución e incidencia en los últimos años. *Phytoma España* 326: 20-27.

García-Martínez O, A Urbaneja, F Ferragut, F Beitia, M Pérez-Hedo (2019c). Persimmon orchards harbor an abundant and well-established predatory mite fauna. *Experimental and Applied Acarology* 77 (2). DOI: 10.1007/s10493-019-00347-7.

Generalitat Valenciana (GVA) (2019). Estadisticas Agricolas, Superficies y producciones de la Comunitat Valenciana. URL: http://agroambient.gva.es/es/estadistiques-agricoles, accessed on 02 May 2021.

Hernández-de la Fuente I, (2018). Estudios preliminares sobre el control de *Dialeurodes citri* (Ashmead) (Hemiptera: Aleyrodidae), por medio de ácaros fitoseidos, en cultivo de caqui en la Comunidad Valenciana. Trabajo Fin de Grado (Grado en Biología), Facultad de Biología, Universidad de Salamanca, Salamanca, 29pp.

Hernández-De la Fuente I, M Laurín, F Beitia, J Tormos (2019). Estudio preliminar sobre la evolucion poblacional de *Dialeurodes citri* (Ashmed) (Hemiptera: Aleyrodidae), en cultivos de caqui en la Comunidad Valenciana. *Agrícola Vergel* 422: 219-226.

Instituto Nacional de Estadística (INE) (2019). Anuario de Estadístico de España 2019. URL: https://www.ine.es/prodyser/pubweb/anuario19/anu19_12agric.pdf, accessed on 02 May 2021.

Intrigliolo DS, L Bonet, P Ferrer, C Besada, A Salvador (2011). Short-Term Effects of Regulated Deficit Irrigation of ‘Rojo Brillante’ Persimmon (Dyospyros kaki) – Yield, Fruit Quality and Post-Harvest Performance. *Acta Horticulturae* 922: 113-120.

Itamura H, Q Zheng, K Akaura (2005). Industry and research trend of Japanese persimmon. *Acta Horticulturae* 685:37–44.

Martínez-Las Heras R, JC Amigo-Sánchez, A Heredia, ML Castelló, A Andrés (2018). Influence of preharvest treatments to reduce the seasonality of persimmon production on color, texture and antioxidant properties during storage. CyTA-Journal of Food 14(2): 333-339.

Medha KG, P Awasthi, A Pandey (2019). Value added preserved food product from persimmon (*Diospyrus kaki*) fruit. *International Journal of Innovative Agriculture* 8(1): 66-71.

Monzó C., PA Stansly (2016). Consequences of Asian citrus psyllid intensive insecticide management strategies on phytoseiid mite assemblages. Bulletin IOBC-WPRS, 120: 32-35.

Monzó C, PA Stansly (2020). Economic value of conservation biological control for management of the Asian citrus psyllid, vector of citrus Huanglongbing disease. *Pest Management Science* 76 (5): 1691-1698.

Llacér G, ML Badenes (2009). Production of Persimmon in Spain. *Acta Horticulturae* 833:39-42.

Perucho R (2015). El cultivo del caqui. Antecedentes e importancia económica. In: El cultivo del caqui, M.L. Badenes, D.S. Intrigliolo, A. Salvador and A. Vicent, eds. (Generalitat Valenciana), pp: 17-34.

R Development Core Team. 2019. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3- 900051-07-0, Available via dialog http://www.R-project.org, accessed 07 July 2020

Salman SY, F Aydinili, RR Ay (2015). Etoxazole resistance in predatory mite *Phytoseiulus persimilis* A.-H. (Acari: Phytoseiidae): Cross-resistance, inheritance and biochemical (Acari: Phytoseiidae): Cross-resistance, inheritance and biochemical resistance mechanisms. *Pesticide Biochemistry and Physiology* 122: 96-102.

Tena A, M Pérez-Hedo, J Catalán, M Juan-Blasco, A Urbaneja (2015). Fitófagos plaga asociados al cultivo del caqui. In: El cultivo del caqui, Generalitat Valenciana (ed) Valencia (Spain), pp: 207–240 (ISBN: 978-84-482-6018-7).

Tixier, MS (2018). Predatory Mites (Acari: Phytoseiidae) in Agro-Ecosystems and Conservation Biological Control: A Review and Explorative Approach for Forecasting Plant-Predatory Mite Interactions and Mite Dispersal. *Frontiers in Ecology and Evolution.* DOI: 10.3389/fevo.2018.00192.

Yonemori K, C Honsho, A Kitajima, M Aradhya, E Giordani, E Bellini, DE Parfitt (2008). Relationship of European persimmon (*Diospyros kaki* Thub.) cultivars to Asian cultivars, characterized using AFLP. *Genetic Resources and Crop Evolution* 55: 81-89.

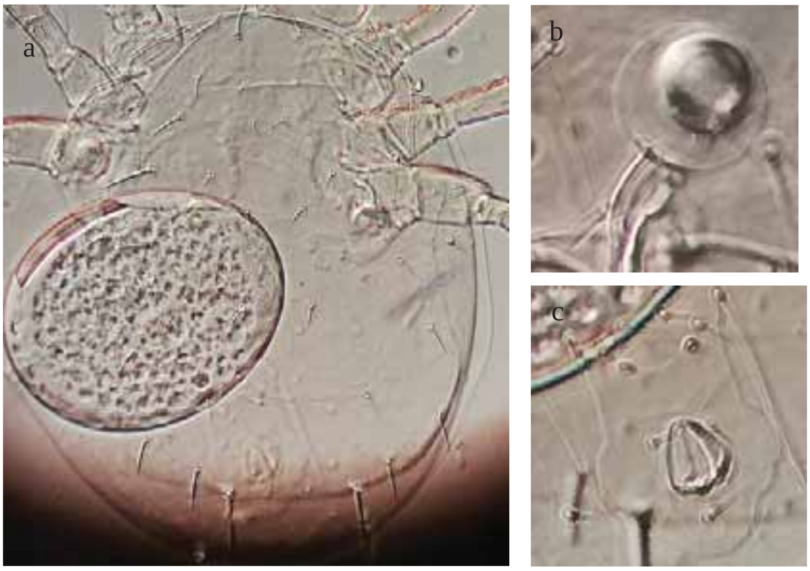
Yokozawa T, YA Kim, HY Kim, YA Lee, G Nonaka (2007). Protective effect of persimmon peels polyphenol against high glucose-induced oxidative stress in LLC-PK1 cells. *Food Chemical Toxicology* 45:1979–1987

Xie C, Z Xie, X Xu, D Yang (2015). Persimmon (*Diospyros kaki* L.) leaves: A review on traditional uses, phytochemistry and pharmacological properties. *Journal of Ethnopharmacology* 163: 229-240. DOI: 10.1016/j.jep.2015.01.007.

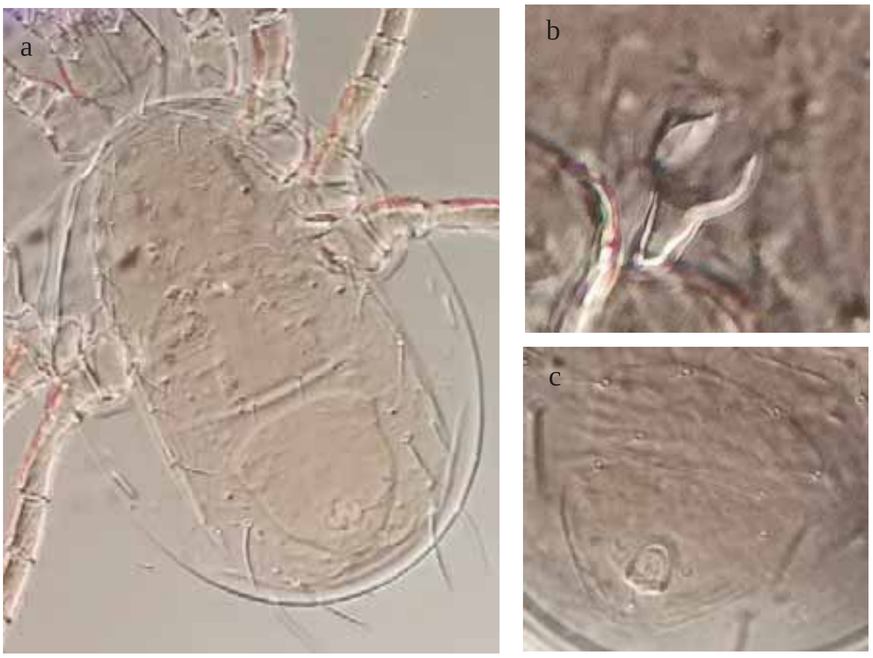
Yuniastuti E, MAA Saputro, Nandariyah, MNI Delfianti (2021). Characterization of persimmon (Diospyros kaki L.) as biodiversity originated from Indonesia. IOP Conf. Series: Earth and Environmental Science 824 (2021) 012040. DOI:10.1088/1755-1315/824/1/012040.

Žanić K, JI Barčić, S Kačić (2001). *Dialeurodes citri* (Ashmead, 1885) in the Adriatic Region of Croatia. *Agriculturae Conspectus Scientificus* 66(3): 161-168.

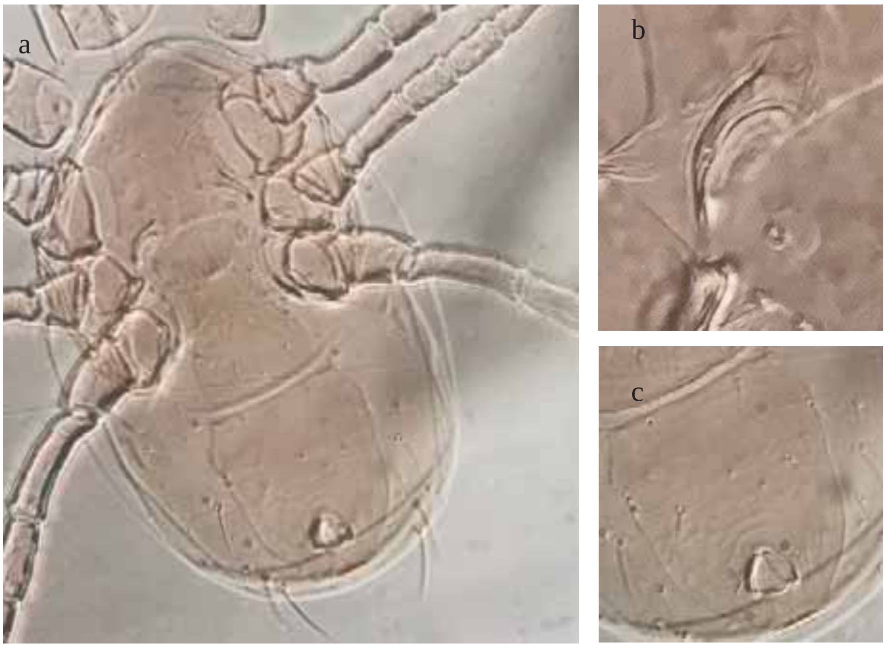
7. ANNEX



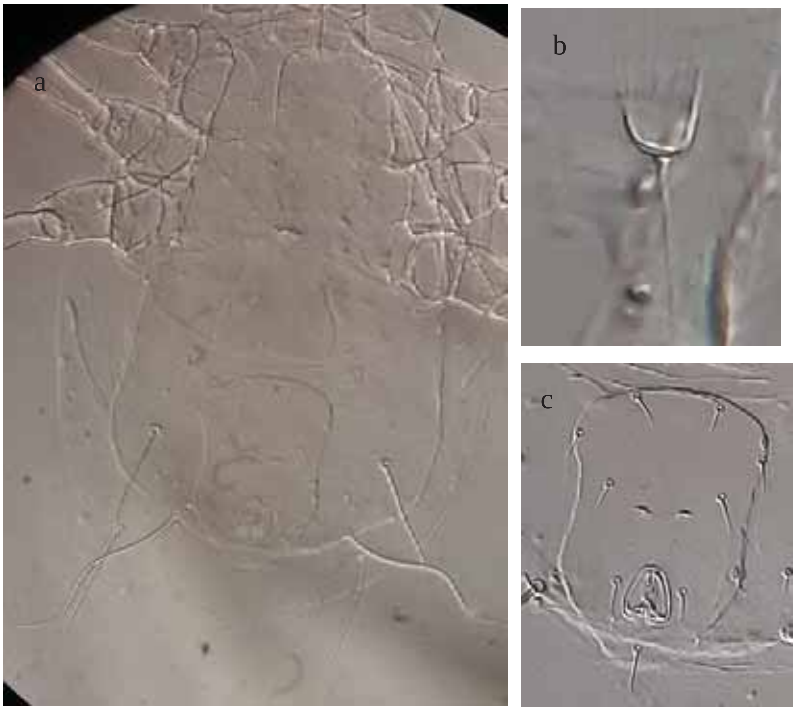
Annex Figure 1. *Euseius stipulatus* (a) dorsal shield, (b) spermatheca, (c) ventrianal plate



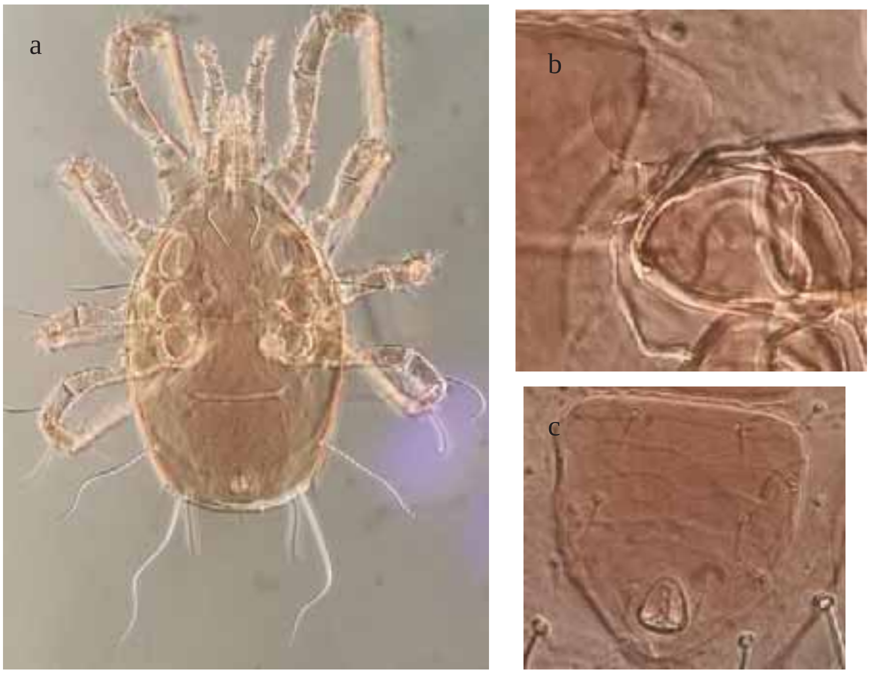
Annex figure 2. *Typhlodromus phialatus* (a) dorsal shield, (b) spermatheca, (c) ventrianal plate



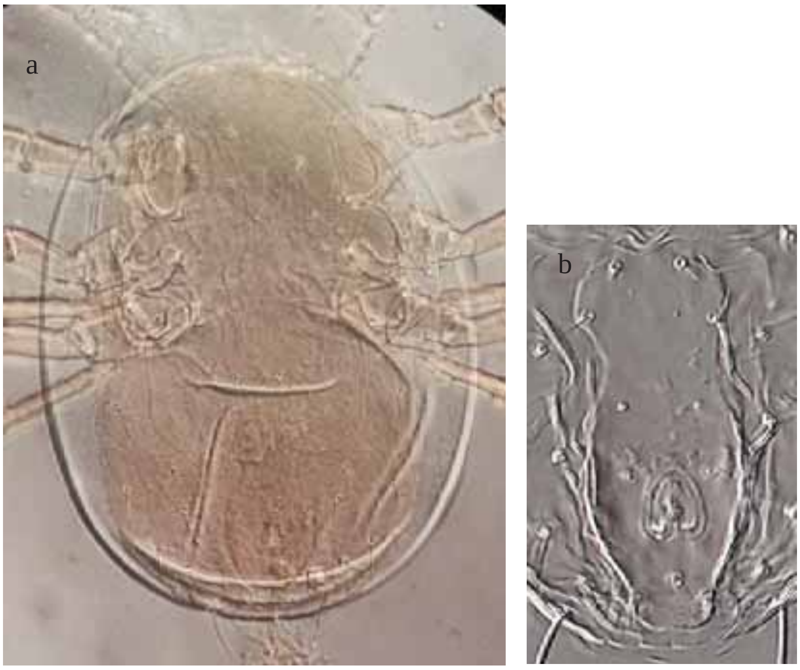
Annex figure 3. *Neoseiulus barkeri* (a) dorsal shield, (b) spermatheca, (c) ventrianal plate



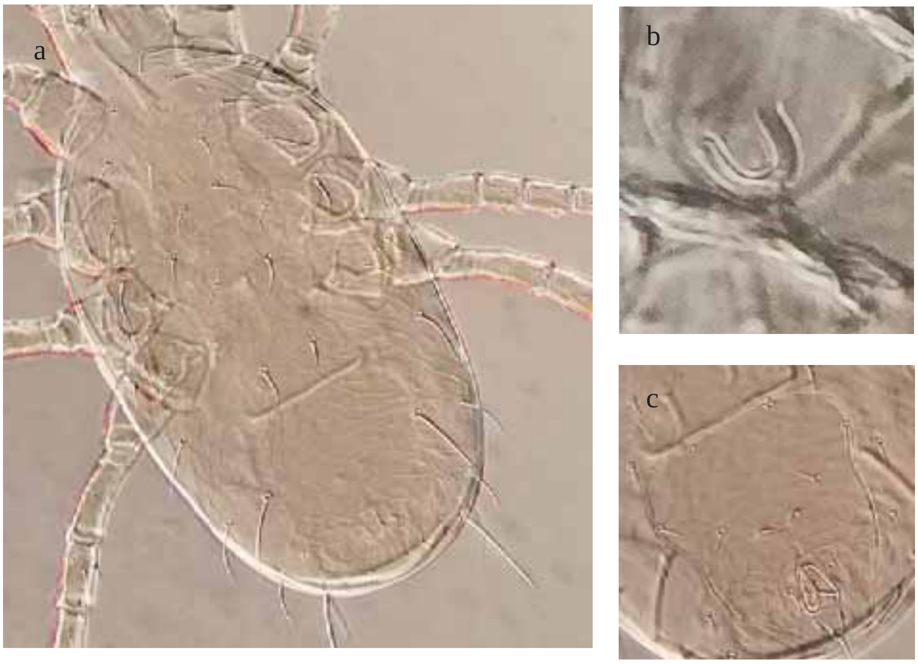
Annex figure 4. *Amblyseius andersoni* (a) dorsal shield, (b) spermatheca, (c) ventrianal plate



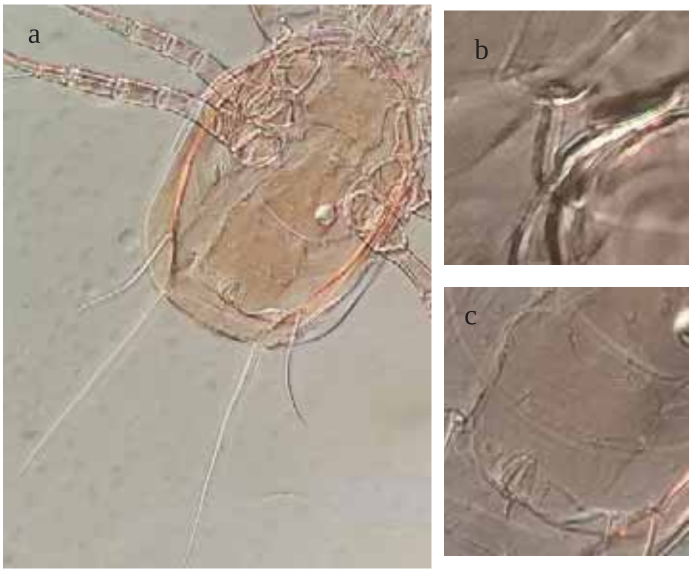
Annex figure 5. *Proprioseiopsis messor* (a) dorsal shield, (b) spermatheca, (c) ventrianal plate



Annex figure 6. *Paraseiulus talbii* (a) dorsal shield, (b) ventrianal plate



Annex figure 7. *Neoseiulus californicus* (a) dorsal shield, (b) spermatheca, (c) ventrianal plate



Annex figure 8. *Amblyseius obtusus* (a) dorsal shield, (b) spermatheca, (c) ventrianal plate

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