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Sustainable weed management and predatory mite (Acari: Phytoseiidae) dynamics in Tunisian citrus orchards

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ABSTRACT — The impact of agroecological weed management on predatory mites (Mesostigmata: Phytoseiidae) is more and more studied. Based on surveys carried out in two experimental sites in Tunisian citrus orchards, this study aims: (i) to compare Phytoseiidae communities on citrus trees and weeds, (ii) to determine dispersal between agrosystem compartments and (iii) to assess the impact of weed management on Phytoseiidae communities. Samples were collected on trees and weeds; dispersal between ground cover and trees was surveyed using traps along tree trunks. *Euseius stipulatus* and, to a lesser extent, *Iphiseius degenerans* were the main species on citrus trees. Phytoseiidae were observed in weeds, with diversity and densities varying according to plant species. Phytoseiidae species in weeds were globally similar to those observed on trees. Phytoseiidae were trapped along the trunk; however higher dispersal was observed from weeds to trees than from trees to weeds. In one survey, species moving up the trees were not the same as those present on trees. With respect to weed management strategies, it seems that ploughed plots favour Phytoseiidae mite dispersal from weeds to trees with consequent increases in densities on trees. This weeding strategy therefore requires more attention to determine how its schedule can enhance biological control.

KEYWORDS — Phytoseiidae; dispersal; weeds; biodiversity; agroecology

INTRODUCTION

Biological control is a key crop protection strategy to limit pesticide use (Wilson and Huffaker 1976). It is considered in some extent (conservation biological control) as an ecosystem service ensuring sustainable production (Crowder and Jabbour 2014). Many studies report positive effect of agrosystem diversification on natural enemy due to more continuous resource availability (preys/host, pollen, nectar, micro-habitats) (*i.e.* Root 1973; Altieri 1999; Landis *et al.* 2000; Landis and Wratten 2002; Wezel

and Soldat 2009; Ratnadass *et al.* 2012; Philpot 2013). The present study focuses on the effect of weed management in citrus orchards on Phytoseiidae mite communities. These biological agents are efficient predators in controlling phytophagous mites and small insects in various crops worldwide (McMurtry and Croft 1997; McMurtry *et al.* 2013). Most of the 2,600 species (Chant and McMurtry 2007; Demite *et al.* 2014) are generalist predators, able to develop feeding on a wide range of foods including small arthropods, pollen, plant exudates and fungi (McMurtry and Croft 1997; McMurtry *et*

al. 2013). Impact of plant leaf architecture (domatia, hairiness) on their development is also well documented for various species (*i.e.* Walter 1992; Karban *et al.* 1995; Kreiter *et al.* 2002b; Villanueva and Childers 2006; Tixier *et al.* 2007; Schmidt 2014). Several studies focusing on perennial crops report the impact of neighbouring vegetation on Phytoseiidae density and diversity (*i.e.* Tuovinen and Rokx 1991; Coli *et al.* 1994; Tuovinen, 1994; Barbar *et al.* 2006; Tixier *et al.* 2006). Some other studies also focus on the effect of plant management diversity within crops, *i.e.* weed management, on Phytoseiidae abundance and diversity (Rock and Yeargan 1973; Hislop and Prokopy 1981; Smith and Papacek 1991; Kreiter *et al.* 1993; Liang and Huang 1994; Nyrop *et al.* 1994; Stanyard *et al.* 1997; Grafton-Cardwell *et al.* 1999; Pereira *et al.* 2006; Aguilar-Fenollosa *et al.* 2008, 2011; Mailloux *et al.* 2010; De Villiers and Pringle 2011). All of them show that weeds are reservoirs of Phytoseiidae; however, only some concern citrus orchards (Liang and Huang 1994; Grafton-Cardwell *et al.* 1999; Aguilar-Fenollosa *et al.* 2008, 2011; Mailloux *et al.* 2010). Furthermore, few studies show effect of weeds on pest and Phytoseiidae migration from ground cover to crop trees (Johnson and Croft 1981; Meagher and Meyer 1990; Flexner *et al.* 1991; Kreiter *et al.* 1991; Alston 1994; Jung and Croft 2001). Herbicide application has for instance been reported to increase dispersal of pest mites from weeds to trees because of habitat destruction (Flexner *et al.* 1991; Kreiter *et al.* 1991, 1993; Alston 1994; Hardman *et al.* 2005, 2011).

The present study thus aims to determine the effect of weed management on: (i) Phytoseiidae communities in the ground cover and citrus trees, and (ii) Phytoseiidae dispersal between weeds and trees.

MATERIALS AND METHODS

Experimental sites. The experiments have been carried out in two sites with different weed management modalities and citrus species. Both experimental sites are located in dry to semi-arid climatic region marked by irregular precipitations. No pesticide was applied during experiments in both sites.

The experimental site 1 is an 11 years-old citrus orchard (*Citrus clementina* Hort. ex Tanaka var. Marisol) of about 2000 m², located in Boumhel, region of Morneg, Cap-Bon, Tunisia (36.72°N, 10.32°E). Four modalities of weed management were considered, with four replicates (nine trees each) of each modality: Mod.1, spontaneous natural vegetation; Mod.2, weeds once sprayed with the herbicide glyphosate on 02-VI-2011; Mod.3, mown weeds once on 02-VI-2011; Mod.4, ploughed ground once on 02-VI-2011 (Figure 1a).

The experimental site 2 is a 20 years-old citrus orchard of about 1 ha located at Sidi Thabèt in the North of Tunisia (36.90°N, 10.04°E). It is planted with three citrus species (*Citrus sinensis* (L.) Osbeck var. Maltaise, *C. clementina* var. Marisol and *C. limetta* Risso var. Limette douce de Tunisie). Two weed management modalities were considered with two replicates (ten trees each) per citrus species: spontaneous natural vegetation and ploughed ground once on 01-VI-2011 (Figure 1b).

Mite samplings and identification. Samplings were carried out once a week from 02-VI-2011 to 18-VIII-2011 for the experimental site 1 and from 11-V-2011 to 30-VII-2011 for the experimental site 2. Thirty citrus leaves per replicate were randomly collected on trees. Weeds included in a quadrat (30 x 30 cm) haphazardly defined were all collected in the groundcover of each replicate. Citrus leaves and weeds were transported in plastic bags in freezing boxes to the laboratory. Mites were then collected and counted: (i) from each citrus leaf using a fine hair brush and (ii) from weeds using the dipping-checking-washing-filtering method (Boller 1984). All Phytoseiidae were mounted on slides using Hoyer's medium for further identification with a phase contrast microscope (Leica DLMB, Leica Microsystemes SAS, Rueil-Malmaison, France). The generic classification of Chant and McMurtry (2007) and other specific literature for species identification were used (Ferragut *et al.* 2009; Papadoulis *et al.* 2009). Mites of the family Tetranychidae were counted but not identified at species level.

Characterisation of Phytoseiidae dispersal. Phytoseiidae ambulatory dispersal between weeds and citrus trees was assessed using traps installed

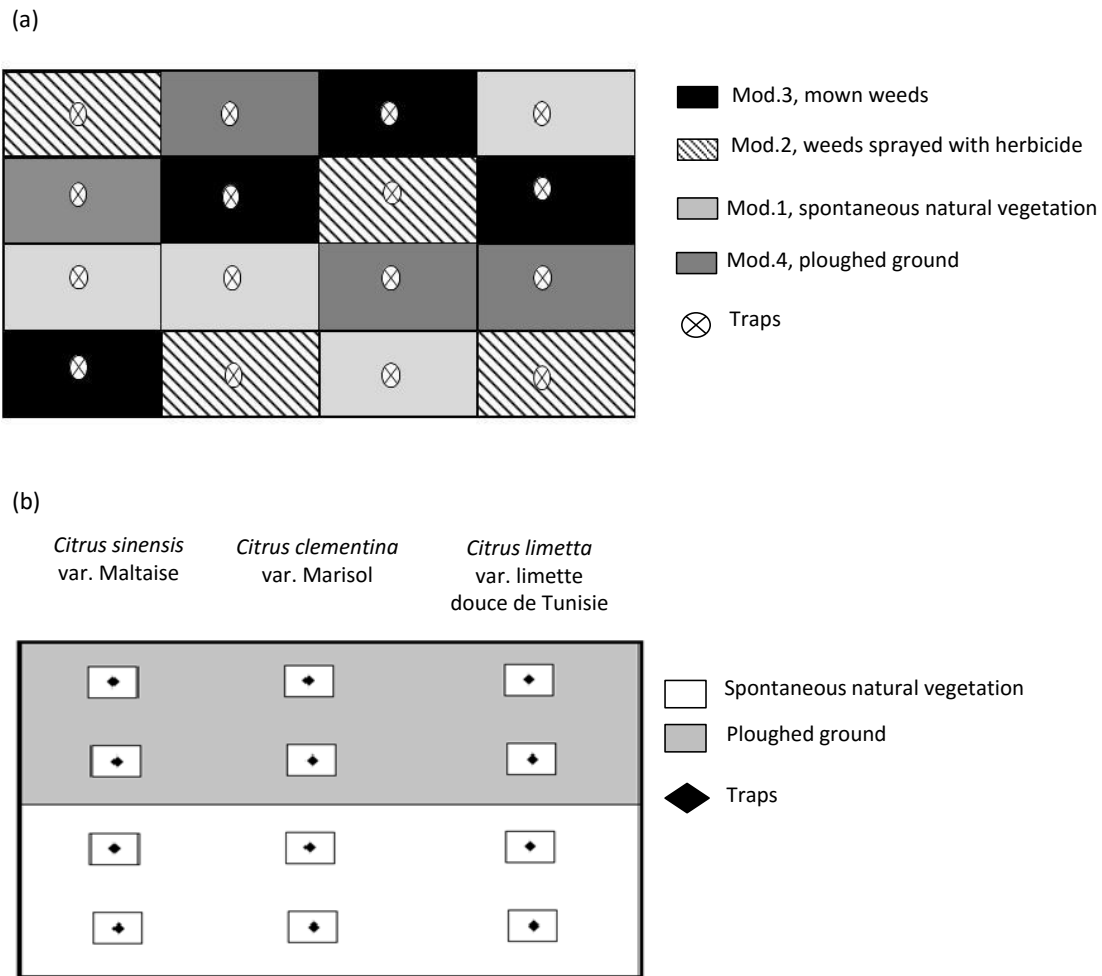


FIGURE 1: Experimental design of the experimental sites 1 (a) and 2 (b).

on 03-VI-2011 (experimental site 1) and 12-V-2011 (experimental site 2). Traps were similar to those used by Koike and Nemoto (2000) for studying Phytoseiidae overwintering (Koike and Nemoto 2000; Kawashima and Amano 2006; Toyoshima *et al.* 2006). They consist in black wool yarn attached to a Velcro®'s hooked surface, all of this stapled on a vinyl- tape (Figure 2). A glue transversal line was applied in the middle of the trap to distinguish between Phytoseiidae moving upwards and downwards. One trap per replicate was placed around the trunk of a randomly selected tree. Once a week, the traps were brought back to the lab, directly ob-

served using a stereoscopic microscope and mites were counted and collected for identification.

Statistical analyses. The thirty citrus leaves and each weed quadrat were considered as replicates to compare Phytoseiidae densities between the modalities considered. Each trap represents a replicate to compare the captured Phytoseiidae numbers depending on the weed management and the dispersal direction. First data normality and variance homogeneity were tested. On case one of these two conditions were not filled: (i) non parametric ANOVA (Kruskal-Wallis) and post-hoc mean comparisons tests were carried to compare the four

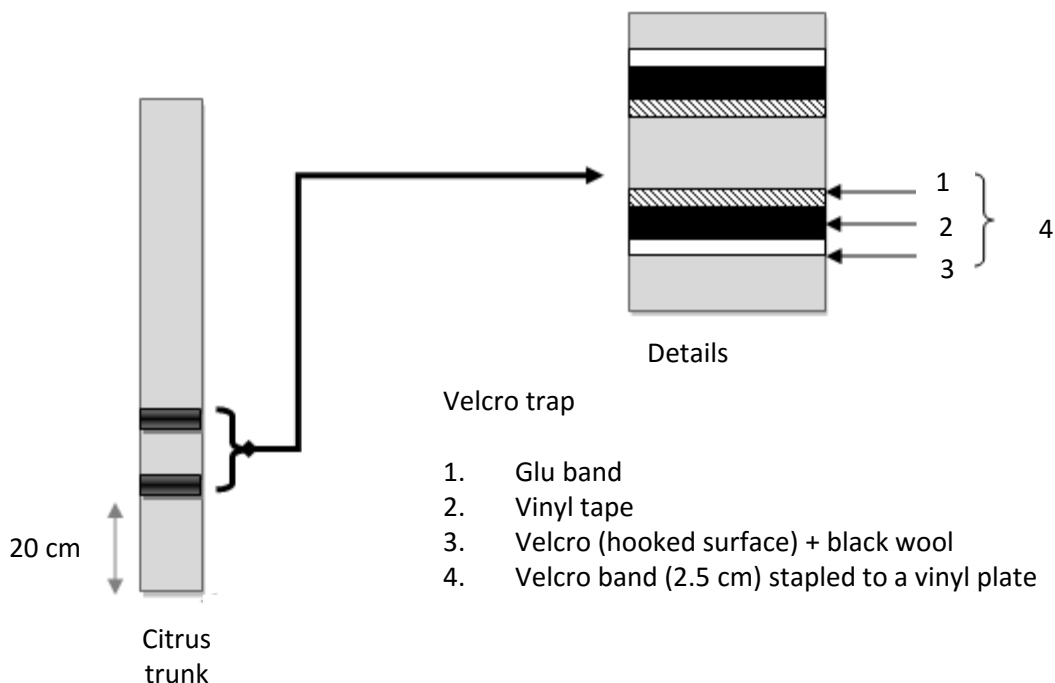


FIGURE 2: Schemas of ambulatory traps used in the experimental sites 1 and 2.

modalities in the experimental 1 (providing *H* values), and (ii) Mann & Whitney non parametric comparison tests were carried out to compare the two modalities in the experimental 2 (providing *Z* values). This latter statistical test was also used to compare densities moving upwards and downwards in the two experimental sites. Linear regression tests between Phytoseiidae mean densities in citrus trees, in weeds and traps were carried out. All the statistical analyses were performed using Statistica version 9 (Statsoft 2010).

RESULTS

Experimental site 1

Phytoseiidae on citrus trees. Three Phytoseiidae species were found on citrus trees: *Euseius stipulatus* (Athias-Henriot), *Neoseiulus californicus* (McGregor) and *Typhlodromus (Anthoseius) rhenanoides* Athias-

Henriot (Table 1); the most abundant was *E. stipulatus* whatever the weeding management. Considering the overall dataset, mean densities were significantly lower in the Mod.2 (herbicide treatment) than in the three others ($H_{(3, 4800)} = 15.30, P = 0.001$) (Figure 3a). These differences were observed on 09-VI ($H_{(3, 4800)} = 23.12, P = 0.00$) and 23-VI ($H_{(3, 4800)} = 11.27, P = 0.01$). After this latter date, Phytoseiidae densities decreased in all the modalities, and no more significant difference was observed.

Phytoseiidae on weeds. Eight Phytoseiidae species were collected on weeds; two are new for the Tunisian fauna (Kreiter *et al.* 2010): *Typhlodromus (Anthoseius) pegazzani* Ragusa and Swirski and *Amblyseius meridionalis* Berlese (Tables 1, 2). *Euseius stipulatus* clearly dominates whatever the weed management strategy; *N. californicus* and *T. (A.) rhenanoides* are the second and third prevalent species on weeds. *Euseius stipulatus* was found on

TABLE 1: Diversity (%) of Phytoseiidae observed on citrus trees, weeds and caught in ambulatory traps in the different modalities of the two experimental sites.

| | Experimental site 1 | | | | Experimental site 2 | | | | | |
|--|----------------------------------|-------------------------|--------------|-------------------|--------------------------------|-----------------|--------------------------------|-----------------|--------------------------------|-----------------|
| | Mod.1 | Mod.2 | Mod.3 | Mod.4 | C. sinensis 'maltaise' | | C. clementina | | C. limetta | |
| | "spontaneous natural vegetation" | "herbicide application" | "mown weeds" | "ploughed ground" | spontaneous natural vegetation | ploughed ground | spontaneous natural vegetation | ploughed ground | spontaneous natural vegetation | ploughed ground |
| Citrus tree | | | | | | | | | | |
| <i>Euseius stipulatus</i> | 98 | 94 | 97 | 98 | 67 | 83 | 74 | 90 | 1 | 52 |
| <i>Iphiseius degenerans</i> | -- | -- | -- | -- | 4 | 1 | 4 | 3 | 86 | 26 |
| <i>Neoseiulus californicus</i> | 0 | 6 | 3 | -- | -- | -- | -- | -- | -- | -- |
| <i>Neoseiulus cucumeris</i> | -- | -- | -- | -- | 0 | 0 | 2 | 0 | 0 | 0 |
| <i>Typhlodromys (Anthoseius) rhenanoides</i> | 2 | 0 | 0 | 2 | 0 | 0 | 4 | 0 | 0 | 0 |
| <i>Typhlodromus (Typhlodromus) exhilaratus</i> | -- | -- | -- | -- | 28 | 13 | 12 | 7 | 11 | 19 |
| <i>Typhlodromus (Typhlodromus) phialatus</i> | -- | -- | -- | -- | 0 | 2 | 4 | 0 | 1 | 2 |
| weeds | | | | | | | | | | |
| <i>Euseius stipulatus</i> | 51 | 64 | 68 | 73 | 11 | 32 | 12 | 15 | 2 | 9 |
| <i>Iphiseius degenerans</i> | -- | -- | -- | -- | 4 | 0 | 15 | 0 | 66 | 14 |
| <i>Neoseiulus barkeri</i> | -- | -- | -- | -- | 7 | 0 | 0 | 0 | 0 | 0 |
| <i>Neoseiulus californicus</i> | 33 | 14 | 18 | 7 | 0 | 1 | 3 | 9 | 0 | 0 |
| <i>Neoseiulus cucumeris</i> | 2 | 0 | 0 | 0 | 11 | 2 | 0 | 3 | 1 | 4 |
| <i>Neoseiulus longilaterus</i> | -- | -- | -- | -- | 7 | 1 | 1 | 3 | 2 | 1 |
| <i>Neoseiulus paspalivorus</i> | -- | -- | -- | -- | 0 | 0 | 0 | 0 | 0 | 1 |
| <i>Amblyseius meridionalis</i> | 0 | 0 | 0 | 7 | -- | -- | -- | -- | -- | -- |
| <i>Phytoseiulus persimilis</i> | 0 | 0 | 0 | 13 | 15 | 0 | 24 | 4 | 0 | 0 |
| <i>Typhlodromys (Anthoseius) rhenanoides</i> | 11 | 4 | 14 | 0 | -- | -- | -- | -- | -- | -- |
| <i>Typhlodromus (Typhlodromus) exhilaratus</i> | 0 | 18 | 0 | 0 | 44 | 57 | 42 | 60 | 26 | 56 |
| <i>Typhlodromus (Typhlodromus) phialatus</i> | -- | -- | -- | -- | 0 | 8 | 3 | 5 | 2 | 16 |
| Traps | | | | | | | | | | |
| <i>Euseius stipulatus</i> | 40 | 60 | 66 | 87,5 | -- | -- | -- | -- | -- | -- |
| <i>Neoseiulus californicus</i> | 0 | 20 | 17 | 0 | -- | -- | -- | -- | -- | -- |
| <i>Typhlodromys (Anthoseius) rhenanoides</i> | 60 | 0 | 17 | 12 | -- | -- | -- | -- | -- | -- |
| <i>Typhlodromys (Anthoseius) pegazzani</i> | 0 | 20 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 56 |
| <i>Typhlodromus (Typhlodromus) phialatus</i> | -- | -- | -- | -- | 100 | 0 | 0 | 0 | 83 | 22 |
| <i>Typhlodromys (Anthoseius) foenilis</i> | -- | -- | -- | -- | 0 | 0 | 0 | 0 | 17 | 22 |

nine plants with the highest densities observed on *Amaranthus retroflexus* L. (Amaranthaceae). *Neoseiulus californicus* was collected on seven plants and was particularly abundant on *Cynodon dactylon* (L.) Persoon (Poaceae). Finally, *T. (A.) rhenanoides* was collected on five plants (Table 2). Although the total number of Phytoseiidae collected during all the experiment was higher in the modalities wild cover (Mod.1) and mown weeds (Mod.3), mean densities were not statistically different between the four modalities ($H_{(3, 160)} = 7.41, P = 0.06$) (Figure 3b). No significant difference was either observed at each date.

Phytoseiidae captured. The three most abundant species found on trees and weeds were also caught; the most captured was *E. stipulatus* (67 %) whatever the weeding modality (Table 1). Species dispersing downwards and upwards were the same. Female was the most captured stage (75 %) followed by immature (21 %) and males

(4 %). Higher densities were caught in the direction "weeds to tree" than in the direction "tree to weeds" (Figure 3c) ($H_{(1,32)} = 8.07, P = 0.004$). Considering each modality separately, this trend was only significant for the Mod.1 (wild cover) ($H_{(1, 80)} = 4.97, P = 0.026$) and the Mod.4 (ploughed ground) ($H_{(1, 80)} = 5.26, P = 0.021$). In this latter modality, the mean number of Phytoseiidae moving upwards was significantly higher than in the other modalities ($H_{(3, 160)} = 23.29, P < 0.01$), especially at 09-VI ($H_{(1, 32)} = 5.38, P = 0.02$) and 16-VI ($H_{(1, 32)} = 5.56, P = 0.01$). Phytoseiidae densities dispersing from trees to the weeds were not significantly different between the four modalities ($H_{(3, 160)} = 2.25, P = 0.52$).

Relationships between Phytoseiidae densities on trees, weeds and in traps. A positive linear significant correlation was observed between Phytoseiidae mean densities on citrus and weeds for the Mod.1 (wild cover) ($R^2 = 0.51, P = 0.018$). Positive

TABLE 2: Number of specimens of Phytoseiidae species collected on weeds in the inter-rows of the citrus orchards studied in two experimental sites.

| | | <i>Amblyseius meridionalis</i> | <i>Euseius stipulatus</i> | <i>Iphiseius degenerans</i> | <i>Neoseiulus barkei</i> | <i>Neoseiulus californicus</i> | <i>Neoseiulus cucumeris</i> | <i>Neoseiulus longilaterus</i> | <i>Neoseiulus paspalivorus</i> | <i>Phytoseiulus persimilis</i> | <i>Typhlodromus (A.) rhenanoides</i> | <i>Typhlodromus (T.) exhilaratus</i> | <i>Typhlodromus (T.) phialatus</i> | |
|--------------------------|-------------------------------|--------------------------------|---------------------------|-----------------------------|--------------------------|--------------------------------|-----------------------------|--------------------------------|--------------------------------|--------------------------------|--------------------------------------|--------------------------------------|------------------------------------|---|
| Experimental site 1 | <i>Amaranthus retroflexus</i> | | 35 | | | 3 | | | | | 2 | | | |
| | <i>Beta arvensis</i> | | 19 | | | | | | | | | | | |
| | <i>Bromus diandrus</i> | | 1 | | | | | | | | | | | |
| | <i>Chenopodium murale</i> | | | | | | | | | | 2 | | | |
| | <i>Convolvulus arvensis</i> | 1 | 8 | | | 4 | | | | | 2 | | | |
| | <i>Conyza canadensis</i> | | 12 | | | 6 | | | | | | | | |
| | <i>Cynodon dactylon</i> | | | | | 12 | 1 | | | | 5 | | | |
| | <i>Hordeum murinum</i> | | | | | 13 | | | | | | | | |
| | <i>Malva sp.</i> | | 1 | | | | | | | | 2 | 5 | | |
| | <i>Mercurialis annua</i> | | 5 | | | | | | | | | | | |
| | <i>Solanum nigrum</i> | | 9 | | | | | | | 2 | | | | |
| | <i>Strobilanthes sp.</i> | | 2 | | | | 2 | | | | | | | |
| | Experimental site 2 | | | | | | | | | | | | | |
| <i>C. limetta</i> | <i>Amaranthus blitum</i> | | 7 | 3 | | | 1 | | | | | 61 | 7 | |
| | <i>Arisarum vulgare</i> | | 1 | 2 | | | | | | | | | | |
| | <i>Beta vulgaris</i> | | | 4 | | | | | | | | | | |
| | <i>Chenopodium murale</i> | | | | | | | 1 | 1 | | | | 2 | |
| | <i>Convolvulus arvensis</i> | | 2 | 11 | | | | | | | | 3 | | |
| | <i>Conyza canadensis</i> | | | | | | | | | | | 1 | 3 | |
| | <i>Cynodon dactylon</i> | | | | | | 4 | 3 | | | | | | |
| | <i>Ecballium Elatrium</i> | | | | | | | | | | | 10 | | |
| | <i>Malva sp.</i> | | | | | | | | | | | 6 | | |
| | <i>Solanum nigrum</i> | | 3 | 75 | | | | | | | | 9 | 2 | |
| | <i>C. clementina</i> | <i>Bromus rigidus Roth.</i> | | 3 | | | 4 | | 2 | | | | 1 | |
| | | <i>Chenopodium murale</i> | | | | | | | | | | | 36 | |
| | | <i>Convolvulus arvensis</i> | | 7 | 1 | | 7 | 3 | | | 34 | | 7 | |
| <i>Conyza canadensis</i> | | | | | | | | | | | | | 4 | |
| <i>Cynodon dactylon</i> | | | | | | | | 1 | | | | 3 | | |
| <i>Hordeum murinum</i> | | | | | | | | | | | | 3 | | |
| <i>Lolium sp.</i> | | | 1 | | | | | 1 | | | | | | |
| <i>Malva sp.</i> | | | 1 | | | | | | | 5 | | 1 | 3 | |
| <i>Mercurialis annua</i> | | | 5 | | | | | | | | | 29 | | |
| <i>Solanum nigrum</i> | | | 16 | 22 | | 3 | | | | 2 | | 34 | 3 | |
| <i>C. sinensis</i> | | <i>Amaranthus blitum</i> | | 2 | | | | | | | | | | 7 |
| | | <i>Bromus hordeaceus</i> | | | | | | | 1 | | | | | |
| | | <i>Chenopodium murale</i> | | 132 | | | 1 | 7 | 4 | | | 225 | 25 | |
| | <i>Convolvulus arvensis</i> | | 11 | | 2 | 2 | 1 | 1 | | 4 | | 8 | 5 | |
| | <i>Conyza canadensis</i> | | | | | | | | | | | 14 | | |
| | <i>Cynodon dactylon</i> | | | | | | 3 | | | | | 1 | | |
| | <i>Ecballium Elatrium</i> | | | | | | | | | | | 9 | | |
| | <i>Emex spinosa</i> | | 13 | | | | | | | | | 7 | | |
| | <i>Lolium sp.</i> | | 1 | | | | | | | | | | | |
| | <i>Mercurialis annua</i> | | | | | | | | | 1 | | 23 | | |
| | <i>Phalaris brachystachys</i> | | | 2 | | | | | | | | | | |
| | <i>Phaseolus vulgaris</i> | | | | | | | | | | | | 2 | |
| | <i>Solanum nigrum</i> | | | | 2 | | | | | 1 | | | 1 | |

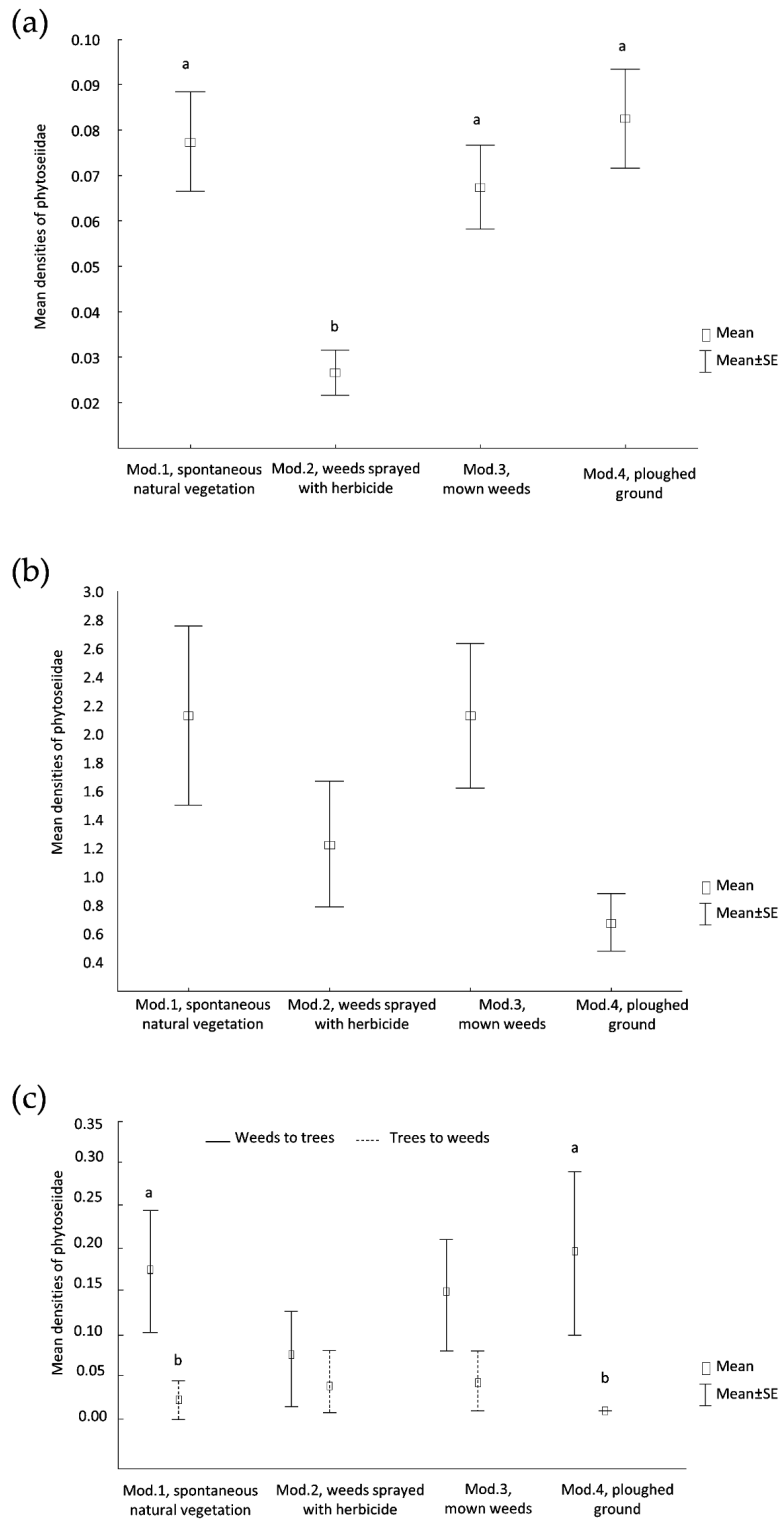


FIGURE 3: Mean Phytoseiidae densities per date (a) per citrus leaf, (b) per weed quadrat and (c) per trap for the four weeding modalities in the experimental site 1.

significant correlations were found between Phytoseiidae mean densities on weeds and those moving upwards in the Mod.3 (mown weeds) ($R^2 = 0.42$, $P = 0.03$) and Mod.4 (ploughed ground) ($R^2 = 0.53$, $P = 0.01$). No significant correlation was observed between Phytoseiidae mean densities in weeds and those moving downwards, whatever the modality considered. A significant correlation between densities on trees and those moving downwards was observed for the Mod.1 (wild cover) ($R^2 = 0.57$, $P = 0.01$). A positive significant correlation was also found between Phytoseiidae mean densities on trees and those moving upwards for the Mod.2 (glyphosate) ($R^2 = 0.59$, $P < 0.01$) and Mod.4 (ploughed ground) ($R^2 = 0.59$, $P < 0.01$) (Table 3).

Experimental site 2

Phytoseiidae on citrus trees. Seven species were observed on citrus; the most abundant was *E. stipulatus* (60 %) followed by *Iphiseius degenerans* (Berlese) (22 %) and *Typhlodromus (Typhlodromus) exilaratus* Ragusa (14 %) (Table 1). On *C. sinensis* and *C. clementina*, the prevalent species was *E. stipulatus* both in ploughed and not ploughed plots (Table 1). On *C. limetta*, *E. stipulatus* was dominant in the ploughed modality and *I. degenerans* in the non-ploughed one. However, no effect of weed management could be concluded, as in both modalities the fauna composition did not change after the ploughing date (1st of June). Phytoseiidae densities were not significantly different between the three citrus species considered ($H(2, 354) = 0.79$, $P = 0.46$) (Figure 4a). When pooling data for the three citrus species, Phytoseiidae mean densities were significantly higher when weeds were ploughed than when they were not ($Z = -2.53$, $P = 0.011$). Such significant effect was however only observed for *C. sinensis* ($Z = -2.73$, $P = 0.006$) (for *C. clementina* $Z = -0.66$, $P = 0.50$, for *C. limetta* $Z = -0.99$, $P = 0.32$) (Figure 4a). During three weeks after ploughing, Phytoseiidae mean densities in ploughed plots were significantly higher than in non-ploughed ones. For *C. sinensis* this effect was observed on 06-VI-2011 ($Z = -0.96$, $P = 0.04$) and 22-VI-2011 ($Z = -2.43$, $P = 0.012$), for *C. clementina* on 06-VI-2011 ($Z = -2.64$, $P = 0.008$) and for *C. limetta* on 14-VI-2011 ($Z = -2.03$, $P = 0.04$).

Phytoseiidae on weeds. The seven species found on citrus trees were also observed on weeds, besides four others: *Phytoseiulus persimilis* Athias-Henriot, *N. californicus*, *Neoseiulus longilaterus* (Athias-Henriot) and *N. paspalivorus* (De Leon). Even if the three prevailing species on weeds were globally the same as on citrus (*E. stipulatus*, *I. degenerans*, *T. (T.) exilaratus*), the most frequent and abundant was *T. (T.) exilaratus* (52 %) (Table 1). On *C. limetta* the proportion of *T. (T.) exilaratus* on weeds was however similar to that of *I. degenerans* (39 and 40 %, respectively). *Typhlodromus (T.) exilaratus* was found on 13 plant species and was particularly abundant on *Chenopodium murale* L. (Amaranthaceae) and in a lesser extent on *Amaranthus retroflexus* L. (Amaranthaceae) and *Solanum nigrum* L. (Solanaceae) (Table 2). *Euseius stipulatus* was found on 12 plant species and was particularly abundant on *C. murale* and in a lesser extent on *S. nigrum*, *Emex spinosa* L. (Campd.) (Polygonaceae) and *Convolvulus arvensis* L. (Convolvulaceae). *Iphiseius degenerans* was reported on five plants and was particularly abundant on *S. nigrum* and in a lesser extent on *C. arvensis*. Even if the number of Phytoseiidae on weeds was higher in the ploughed modality (Figure 4b), this difference was not significant both when all the citrus species were grouped ($Z = -0.35$, $P = 0.72$) and for every citrus species (*C. sinensis*: $Z = -0.78$, $P = 0.43$; *C. clementina*: $Z = 0.71$, $P = 0.47$; *C. limetta*: $Z = -0.25$, $P = 0.79$).

Phytoseiidae captured. Five Phytoseiidae species were captured in ambulatory traps, no specimen of *E. stipulatus* and *I. degenerans* were caught. The dominant species was *T. (T.) exilaratus* (46 %) for the three citrus species considered in both modalities. All stages were captured (female: 80 %, male: 11 % and immature: 9 %). The fauna composition moving downwards and upwards was different; *T. (A.) peggazani* was the prevalent in the downward direction and *T. (T.) exilaratus* in the upward direction whatever the weeding modality. The mean number of Phytoseiidae dispersing from weeds to trees was significantly higher than that dispersing from trees to weeds in the two weeding modalities (ploughed modality [$Z = 4.80$, $P = 0.02$] and not ploughed modality [$Z = 6.97$, $P = 0.008$]).

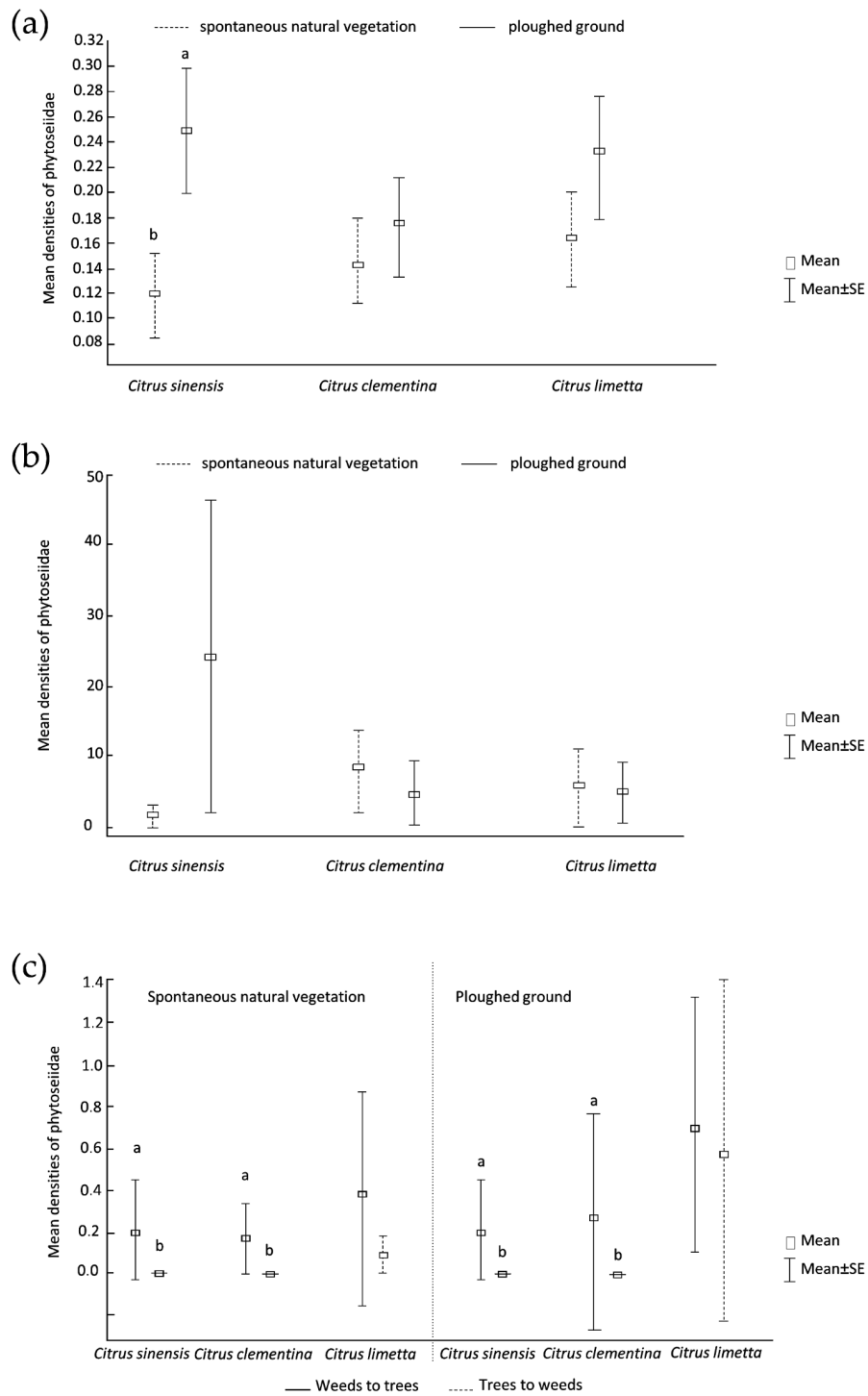


FIGURE 4: Mean Phytoseiid densities (a) per citrus leaf, (b) per weed quadrat and (c) per trap for the two weeding modalities in three citrus species orchards of the experimental 2.

When considering each citrus species, such a difference was only observed for *C. sinensis* ($Z = 6.39$, $P = 0.011$) and *C. clementina* ($Z = 4.15$, $P = 0.04$) (for *C. limetta* [$Z = 2.68$, $P = 0.10$]) (Figure 4c). No global significant effect of weed management was observed on Phytoseiidae mean densities dispersing upwards ($Z = 0.033$, $P = 0.85$) nor downwards ($Z = 0.035$, $P = 0.55$) (Figure 4c). However, the number of Phytoseiidae moving upwards were significantly higher when weeds were ploughed than when they were not on 06-VI and 14-VI, i.e. 6 and 14 days after ploughing ($Z = 29.89$, $P < 0.001$).

Relationships between Phytoseiidae densities on trees, weeds and in traps. A significant linear correlation between Phytoseiidae mean number on citrus trees and weeds ($R^2 = 0.26$, $P = 0.008$) was observed in the modality where weeds were ploughed. However, this correlation was only observed in *C. sinensis* plot ($R^2 = 0.58$, $P = 0.0002$).

DISCUSSION

The Phytoseiidae species on citrus. In the two surveys, the dominant species in citrus was *E. stipulatus* and in a lesser extend *I. degenerans* in one plot of *C. limetta* in the experimental site 2. Both species are common on citrus in the Mediterranean basin even if *E. stipulatus* is usually prevalent (McMurtry 1977; Swirski & Amitai 1990; Ferragut *et al.* 1992; Kreiter *et al.* 2002a; Aucejo *et al.* 2003; Abad-Moyano *et al.* 2009; Aguilar-Fenollosa *et al.* 2011; Tsagkarakis *et al.* 2011; Sahraoui *et al.* 2012; Barbar 2013). Furthermore, only a few females of *Tetranychus* sp. were found on citrus trees. Conclusion on biological control should be however cautious, because: (i) of the low densities of predator, and (ii) *E. stipulatus* is considered by many authors as an herbivore-pollen feeder poorly efficient to penetrate tetranychids webs and control them (Gonzalez *et al.* 2009). The fact that *I. degenerans* was mainly observed on one part of the *C. limetta* orchard whereas *E. stipulatus* prevailed in the other part requires discussion. First, this can be due to high densities of *I. degenerans* on weeds (as *S. nigrum*) in this part of the orchard and subsequent dispersal from weeds and trees. Second, competition on citrus between

I. degenerans and *E. stipulatus* could have affected their respective dominance because of diet differences (Van Rijn and Tanigoshi 1999; Madinelli *et al.* 2002; Vantornhout *et al.* 2004; Villanueva and Childers 2006).

Are weeds reservoirs for Phytoseiidae? In both surveys, Phytoseiidae have been observed on weeds suggesting thus that ground cover constitutes a reservoir for those natural enemies. Phytoseiidae diversity was higher on weeds than on citrus certainly because ground cover provides more diverse and abundant resources (food and habitat) than trees (Tuovinen and Rokx 1991; Barbar *et al.* 2005; Fadamiro *et al.* 2008, 2009). Some plants seem to be more favourable to Phytoseiidae than others (survey 1: *A. retroflexus* for *E. stipulatus*, *C. dactylon* for *N. californicus*; survey 2: *C. murale* and *A. retroflexus* for *T. (T.) exhilaratus* and *E. stipulatus* and *S. nigrum* for *I. degenerans*). Plant leaf architecture but also leaf appetite for such Phytoseiidae generalist predators (McMurtry and Croft 1997; Addison *et al.* 2000; Broufas and Koveos 2000; Kreiter *et al.* 2002a; Bouffras and Papadoulis 2005; Villanueva and Childers 2006; Bermudez *et al.* 2010, Schmidt 2014; Adar *et al.* 2012, 2015) could explain such associations. Besides leaf characteristics, presence of preys (*i.e.* *Tetranychus* spp.) could also explain the abundance of *P. persimilis* a specialist species on *C. arvensis* (McMurtry and Croft 1997) and of *I. degenerans* on *S. nigrum*. Source foods with more accurate identification of preys but also of pollen quantity would thus require more interest for further experiments.

Does the dispersal of Phytoseiidae between citrus trees and inter-rows exist? Aerial dispersal has been tested (data not shown) and any Phytoseiidae have been captured on traps displayed under the canopy. Phytoseiidae ambulatory dispersal between weeds and trees was observed in both surveys, confirming previous results in apple and citrus orchards (Johnson and Croft 1981; Alston, 1994). As in other studies (Johnson and Croft 1981; Koike and Amano 2000), the "normal" sex ratio of mites captured in both surveys seems to reflect that males and females have similar ambulatory dispersal ability. In both surveys the number of Phytoseiidae

dispersing upwards was higher than that moving downwards. Two hypotheses can be proposed to explain such observations: (i) resource foraging (*i.e.* food) and (ii) escape from disturbed habitat. Yet, Phytoseiidae species caught are generalist predators and their dispersal seems to not depend on food foraging (McMurtry and Croft 1997; Jung *et al.* 2001). The second hypothesis seems to be more appropriated, as dispersal was higher when weeds were destroyed (see below).

What this study brings out on relationships between ground cover and citrus trees? The species mainly caught (*E. stipulatus* in survey 1 and *T. [T.] exhilaratus* in survey 2) were also the prevalent species on weeds, suggesting thus a link between Phytoseiidae on weeds and those moving upward along the tree trunks. However, the main species dispersing from weeds to trees did not always prevail on citrus especially in the survey 2 (*E. stipulatus* and *I. degenerans* on citrus and *T. [T.] exhilaratus* in weeds and traps). It seems thus that even if *T. (T.) exhilaratus* reached citrus, it did not settle well on trees. This may be explained by intraguild competition with *E. stipulatus* and/or *I. degenerans*. Abad-Moyano *et al.* (2010a, 2010b) and Aguilar-Fenollosa *et al.* (2011) explain the dominance of *E. stipulatus* in citrus orchards by competitive ability and rapid dispersal on the smooth surfaces of citrus leaves (optimal foraging).

Furthermore, in the survey 2 despite the presence of *E. stipulatus* and *I. degenerans* on weeds these species were not captured moving upwards contrarily to what was observed in the survey 1. This could be due to their low numbers on weeds and to the remote location of those reservoir plants from trees.

For practical outputs, such results underline the importance to manage ground cover for increasing predatory species of interest (*i.e.* *E. stipulatus* and *I. degenerans*) instead of other species that will not succeed to settle on associated trees.

How weed management affects Phytoseiidae communities in citrus, weeds and their dispersal? In the two surveys, weed management modalities did not significantly affect Phytoseiidae diversity nor in weeds nor in trees. It seems thus that cover

crop management at least at short-term is not the key factor determining Phytoseiidae diversity.

Globally in the two surveys, weeding strategies did not affect Phytoseiidae densities in the ground cover. Some tendencies can be however underlined as the lowest Phytoseiidae densities were observed in the modality where herbicide was applied (survey 1). This can be due: (i) to direct lethal effect of glyphosate as observed by Kreiter and Le Menn (1993) and/or (ii) indirect herbicide effects by habitat destruction (Gauvrit 1996).

On citrus, Phytoseiidae densities were not different for the modalities wild cover, mown weeds and ploughed ground in the survey 1. However, densities were much lower when herbicide was applied, suggesting that herbicide in limiting Phytoseiidae in weeds also lead to low densities on trees. This confirms results of Pereira *et al.* (2006) but not those of Aguilar-Fenollosa *et al.* (2011) and Nyrop *et al.* (1994). These divergent results could be due to application period and initial Phytoseiidae densities on weeds and trees, but also to the fact that herbicide application would more reduce pollen quantities than the other weeding strategies.

The highest quantities of Phytoseiidae moving upwards were observed in the modality "ploughed ground" in both surveys. This can be explained by Phytoseiidae escape from "disrupted" environment as emphasized in some studies showing that food and habitat destruction affect Phytoseiidae ambulatory dispersal (Johnson and Croft 1981; Auger *et al.* 1999; Aguilar-Fenollosa *et al.* 2011). Furthermore, also in this latter modality higher densities of Phytoseiidae were observed on trees, suggesting that this weeding strategy could enhance Phytoseiidae migration from ground cover to trees. Finally in both experiments, a time effect of ground cover perturbation was observed; Phytoseiidae densities moving upwards were higher during two weeks after the ploughing.

CONCLUSION

The present experiment provides new insights on the effect of weed management on Phytoseiidae community in citrus agrosystems. Higher diversity

of Phytoseiidae was found on weeds than on crops certainly due to more constant and favourable resources. However the "natural enemy hypothesis" was not completely validated as only one species prevailed on citrus, independently of Phytoseiidae abundance and diversity on weeds. Furthermore, citrus species and weeding practices significantly affected Phytoseiidae densities. Weed ploughing seems to favour Phytoseiidae upwards dispersal with probable subsequent density increase on associated trees. However, the present study presents some limitations, first of all the time period and duration of the experiments and also low densities retrieved overall the experiments.

Second several factors that have not been herein considered could also contribute to an overall comprehension of faunal interactions between agrosystem components. For instance, some authors relate the role of uncultivated areas as pollen provider and alternative food resources for Phytoseiidae (Grafton-Cardwell *et al.* 1999; Duso *et al.* 2004; Maoz *et al.* 2011, 2014; Montserrat *et al.* 2013). Other authors showed studies of interactions between plant and Phytoseiidae should also consider the ability of some species including those belonging to the genus *Euseius* to feed on plants (Adar *et al.* 2012, 2015).

Thus, additional studies including those latter elements as well as a higher number of traps and longer lasting experiments would be required to better understand the overall interaction between Phytoseiidae and their habitats, for finally proposing practical habitat management and enhancing predator efficiency.

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