



Compatibility of organic farming treatments against *Monosteira unicastata* with non-target arthropod fauna of almond trees canopy

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Abstract

Field trials had shown that 1-2 applications of kaolin and potassium salts of fatty acids combined with thyme essential oil (PSTEO) reduced the abundance of the lace bug *Monosteira unicastata* (Mulsant & Rey) (Hemiptera: Tingidae), an important pest of almond trees in the Mediterranean region. These products could be useful for the control of this pest in organic production of almonds, but higher number of applications could be necessary. However, the possible detrimental effects on the almond orchard ecosystem should be evaluated. In the present work, the effects observed on the non-target arthropod fauna of the almond trees canopy in those field assays are shown. First, a comprehensive report of the non-target arthropod fauna of the almond tree is provided. Regarding natural enemies, most of the predatory arthropods captured were spiders belonging to different families like Salticidae, Thomisidae, Philodromidae, Theridiidae, Araneidae or Oxyopidae. Other predatory families that appeared in significant numbers were Chrysopidae, Anthocoridae, Aeolothripidae, Coccinellidae, Phytoseiidae, Erythraeidae or Forficulidae. Among parasitoids, the most abundant families were Eulophidae, Scelionidae and Dryinidae. Kaolin reduced the abundance of natural enemies and other non-target arthropods as well as their diversity and number of species. On the contrary, PSTEO only produced a slight reduction in the number of natural enemies, whereas no effect was found on the diversity and species richness. These effects were observed despite the reduced number of applications, so greater effect is expected if its frequency is increased in order to achieve an efficient control of *M. unicastata*.

Additional keywords: kaolin; insecticidal soaps; thyme essential oil; organic almond production; *Prunus dulcis*; natural enemies

Abbreviations used: PRC (principal response curve analysis); PSTEO (potassium salts of fatty acids combined with thyme essential oil).

Authors' contributions: MGN, ISR and SP designed the research and wrote the manuscript. AM, GC, MGN, ISR and SP sampled arthropods. AM, MGN, ISR, SP and CEF classified arthropods. ISR analyzed the data.

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Introduction

The interest in organic foods grows permanently worldwide, as does the area devoted to this type of agriculture. Organic agriculture land has quadrupled since 1990, reaching a total area of 43.7 million hectares in 2014 (Willer & Lernoud, 2016).

Nuts are among the main permanent organic crops in the world, surpassed only by coffee and olives. In 2014 the worldwide area for organic nuts was 286,109 ha (Willer & Lernoud, 2016) and an important portion of this (94,646 ha) corresponded

to Spain where the main organic nut crop is almond (85,241 ha) (MAGRAMA, 2015).

Monosteira unicastata (Mulsant & Rey) (Hemiptera: Tingidae), commonly known as false tiger or poplar lace bug, is one of the most serious pests of organic almond orchards in Spain (García Mari & Ferragut, 2002; Almacellas & Marín, 2011; Marcotegui *et al.*, 2015). This insect is frequently found damaging almond crops in other Mediterranean countries (Talhouk, 1977; Moleas, 1987; Liotta & Maniglia, 1994; Russo *et al.*, 1994; Bolu, 2007) and it has been cited in North America recently (Scudder, 2012). Adults and nymphs

of this species suck the cellular content of leaves and can cause significant defoliation of trees thus resulting in a yield decrease (Liotta & Maniglia, 1994; García Mari & Ferragut, 2002). These damages are particularly serious in organic almond orchards, where chemical insecticides, commonly used to control this pest, are not authorized.

The production of certified organic food requires practices authorized by organic production standards (IFOAM, 2014; EC, 2015; USDA, 2016). Crop protection in organic agriculture is based on preventive strategies (well adapted varieties, balanced fertility, rotations, companion planting, green manures, functional biodiversity, habitat management, beneficial organisms) and insecticide application is restricted to products of mineral or vegetable origin included in the standards that are used as a last option when prevention has failed (Zehnder *et al.*, 2007). Some studies have been conducted recently to search for products allowed in organic production that would be effective for controlling *M. unicostata* in almond crops. Sánchez-Ramos *et al.* (2014) found out, in laboratory tests, that kaolin sprayed on almond leaves reduced oviposition plus adult and nymphal feeding, while potassium salts of fatty acids combined with thyme essential oil caused high mortality of nymphs. The effect of these products against this pest was also tested in field trials (Marcotegui *et al.*, 2015). In this case, both products reduced abundance of *M. unicostata* and the damage on leaves, being kaolin the most effective.

To conserve or improve biodiversity is a general objective of organic cropping and enhancing abundance and efficacy of the natural enemies existing community is a priority for this production system (Zehnder *et al.*, 2007; IFOAM, 2014). Therefore, the potential impact of any insecticide application on natural enemies arthropod fauna must be previously investigated. Natural biological control of *M. unicostata* has not been deeply studied. Although different groups of predators (like Araneae, Forficulidae, Chrysopidae, Miridae, Anthocoridae, Coccinelidae, Carabidae, and predatory Thysanoptera, Hymenoptera and Diptera) and parasitoids (like Mymaridae) have been reported as possible natural enemies of *M. unicostata* (Vessia, 1961; Moleas, 1987) only Araneae and Anthocoridae have shown an obvious predatory activity against this pest in the field (Moleas, 1987).

Kaolin affects the recognition and attractiveness of host plants by arthropods and has proved to be an effective barrier to prevent damage by phytophagous arthropods in many crops (Showler, 2002; Glenn & Puterka, 2005). Laboratory studies have also reported negative biological and behavioral effects of kaolin particle film on entomophagous arthropods (Ulmer *et*

al., 2006; Porcel *et al.*, 2011; Bengochea *et al.*, 2013, 2014a,b; Benhadi-Marín *et al.*, 2016). In addition, although some field trials did not find negative effects of kaolin on certain natural enemies (Karagounis *et al.*, 2006; Sackett *et al.*, 2007; Porcel *et al.*, 2011) in other studies, populations of different taxa of parasitoid and predatory arthropods were reduced after kaolin applications (Knight *et al.*, 2001; Showler & Sétamou, 2004; Lombardini *et al.*, 2005; Jaastad *et al.*, 2006; Markó *et al.*, 2006, 2008, 2010; Stelinski *et al.*, 2006; Sackett *et al.*, 2007; Pascual *et al.*, 2010; Scalercio *et al.*, 2010).

Insecticidal soaps and plant oils are commonly used for pests control in organic farming (Zehnder *et al.*, 2007) but few data are available on the selectivity of these “organic treatments” to beneficials. No negative effect of insecticidal soaps on natural enemies have been reported in many studies (Natarajan, 1990; Bigler & Waldburger, 1994; Jacas Miret & García-Mari, 2001; Karagounis *et al.*, 2006; Jansen *et al.*, 2010). However, in others, negative effects of soaps applications were found on some groups of natural enemies (Oetting & Latimer, 1995; Smith & Krischik, 2000; Stansly *et al.*, 2002; Kraiss & Cullen, 2008; Raudonis *et al.*, 2010; Hall & Richardson, 2013; Smaili *et al.*, 2014). In the case of essential oils, again some studies reported no negative effect on natural enemies (Echegaray & Cloyd, 2012; González *et al.*, 2013), but some detrimental effects have been also reported (Momen & Amer, 1999; Amer & Momen, 2002; Choi *et al.*, 2004; Bostanian *et al.*, 2005; Huignard *et al.*, 2008; Cloyd *et al.*, 2009).

In the present work, the effects of these organic-farming-compatible pests control products on non-target arthropods of almond tree canopy were evaluated, under real field conditions, paying special attention to the community of natural enemies of pests.

Material and methods

Field trials design

Field trials were conducted in 2009 and 2010 in commercial almond orchards in Murcia, Spain. The experimental design was as described in Marcotegui *et al.* (2015). Briefly, plantations located in Cieza (cultivars Ferragnes and Feraduel) and Cehegín (cultivar Antofñeta) were used in 2009 and 2010 respectively. In both cases the products tested were:

1) Kaolin: Surround® WP (95% w:w (wetable powder) kaolin) (BASF Aktiengesellschaft, Ludwigshafen, Germany) sprayed at a dose of 5 kg/100 L. Two applications were given each year: the first one in mid-spring, aimed to prevent colonization of overwintering

adults of *M. unicastata* and the second one in early summer, to guarantee coating until the end of the crop season.

2) Potassium salts of fatty acids combined with thyme essential oil (PSTEO): OleatBio-to® (40% w:w soybean and sunflower fatty acids; 5% w:w potassium salts; 6% w:w thyme essential oil) (TRABE S.A., Murcia, Spain) was sprayed at a dose of 300 mL/100 L. An application against nymphs of the second generation of *M. unicastata* was given at the end of spring in both years, but in 2010, the populations of the lace-bug were much higher and an additional application was required against the nymphs of the third generation in mid-summer.

3) Unsprayed control.

Products were sprayed at the maximum field recommended concentrations in Spain (De Liñán, 2013a,b). The experimental design consisted of randomised blocks with four (2009) or seven (2010) replications. Within each block, four contiguous trees of each cultivar were randomly assigned to each treatment. See Marcotegui *et al.* (2015) for details.

Assessment of abundance of arthropods

The overall arthropod fauna from the canopy of almond trees was sampled using a beating method. Arthropods sampled from the four trees of each treatment were collected in a plastic bag. Beating sampling was performed monthly in spring and summer, with a total of five and six sampling dates in 2009 and 2010, respectively.

Samples were taken to the laboratory and the specimens were assigned to the following groups:

- 1) Natural enemies: Those belonging to families whose main feeding habit is parasitism or predation.
- 2) Phytophagous (target arthropods): Those causing economic damage on almond trees (pests). This group was studied in Marcotegui *et al.* (2015).
- 3) Other arthropods: Those phytophagous not described as pests on almond trees and specimens with other feeding habits or that could not be allocated to a specific feeding guild.

This work focuses on non-target arthropods, *i.e.* groups 1 and 3. Specimens were determined to family level when possible. Biodiversity was assessed by the number of morphospecies and the Shannon biodiversity index (Magurran, 2004).

Data analysis

The effect of the factors considered on the number of individuals captured, the number of species and the Shannon biodiversity index was tested by linear mixed-effects models (Littell *et al.*, 1998; Wang &

Goonewardene, 2004). Treatment and cultivar (only in 2009) were considered fixed factors, with block as a random factor and sampling date as a repeated measures factor. Interactions among all fixed factors were also considered in the models. The best covariance structure for the repeated-measures (date) factor was selected according to the lowest value of the Akaike and Schwarz's Bayesian information criteria fit statistics (Littell *et al.*, 1998; Wang & Goonewardene, 2004). The models were fitted using a restricted maximum likelihood estimation method. If convergence was not achieved or the final Hessian matrix was not positive definite, the random factor was removed from the model as it was identified as redundant variable. When necessary, data were previously transformed by $\ln(x+1)$ for normality. The significance level was always $p < 0.05$. Statistical tests were performed using SPSS statistical program.

Analyses were performed for the periods before and after the first treatment application. In the first case, to verify the absence of significant differences among plots and in the second case, to examine the effect of the treatments on abundance and diversity of non-target arthropods. Differences in abundance and diversity of non-target arthropods among plots assigned to each treatment were evaluated separately against the untreated control in 2009 because of the different application schedule of kaolin and PSTEO. For 2010 data, differences with regard to the control were established by an LSD test when statistical significance was found.

To investigate changes in abundance and species composition of the non-target arthropod community in the canopy of almond trees, a principal response curve (PRC) analysis was performed using the program CANOCO 4.51 (Van den Brink & Ter Braak, 1999; Leps & Smilauer, 2003). The significance of the deviations from the line representing the untreated control ($y=0$), because of each treatment, was tested using an F-type permutation test (Monte Carlo simulation) with 499 permutations. PRCs for kaolin and PSTEO plots in relation to the untreated controls were obtained before and after the first treatment applications for each year. Additionally, to determine treatment effects on different taxa, 'species weights' were also considered in those cases in which the PRCs were significant. Data on the number of captures of each taxon were transformed to $\ln(x+1)$ before analysis.

Results

Arthropod community in organic almond orchards

In the 2009 orchard, the great majority of arthropods captured were phytophagous potentially harmful

on almond trees (87.5-90%), with the other groups appearing in very small proportions (predators: 3.7-4.7%; parasitoids: 1.9-2.0%; other arthropods: 4.4-5.4%) (Table S1 [suppl.]). In 2010, the situation was different, with phytophagous again as the dominant group (53.8-58.8%), but with the “other arthropods” group showing a much higher proportion (37.2-42.0%). Predators (3.1-4.0%) and parasitoids (0.6-1.0%) were again minor groups. The composition of the community of phytophagous arthropods is described in Marcotegui *et al.* (2015).

Predators

The most abundant predators captured in 2009 were spiders (30-42%) comprising eight families of which Salticidae (9-13%), Philodromidae (4-7%) and Theridiidae (3-6%) were predominant (Table S2 [suppl.]). A small percentage of spiders (3-8%) could not be determined since they were damaged. The next groups in order of abundance were Neuroptera (18-20%) and Hemiptera (17-21%), which appeared in similar proportions. Most of the captured Neuroptera belonged to the family Chrysopidae (17-19%) and the rest were Coniopterygidae, but with very low percentages (<2%). With regard to predatory Hemiptera, Anthocoridae (14-18%) was the dominant family, while Miridae appeared in smaller proportions (<4%). Acari were the next group in abundance (10-16%), represented by six families of predatory mites, of which Phytoseiidae were by far the most abundant (8-11%). Coleoptera (2-9%) mainly represented by Coccinellidae (1-8%), Thysanoptera of the family Aeolothripidae (3-6%) and Dermaptera of the family Forficulidae (3-4%) were the next groups in order of abundance. Predators belonging to Diptera and Dytioptera were the least frequent in samples (<2%).

In 2010, again spiders was the most abundant group, with higher proportions than those observed in the previous year (42-52%). The family composition was very similar to that found in the 2009 orchard, but the relative abundance was somehow different. Thus, in 2010 Thomisidae and Philodromidae (8-14% and ~12%, respectively) were the most abundant families, followed by Theridiidae (5-9%), Araneidae (5-7%), Salticidae (3-6%) and Oxyopidae (3-6%). Like in the previous year orchard, Neuroptera (10-14%) was one of the next most abundant groups, with the family Chrysopidae representing the majority of the individuals captured. However, unlike 2009, this time Neuroptera was tied with predatory Acari (10-15%), being Erythraeidae the dominant family (9-11%), whereas Phytoseiidae appeared in much lower proportions (1-2%). The next groups in order of abundance were Thysanoptera of the family Aeolothripidae (6-11%) and Coleoptera (5-13%), represented by Coccinellidae (5-10%) and Malachiidae

(<4%). Hemiptera (4-7%) appeared this year in lower proportion compared to the previous orchard, but again Anthocoridae was the main family (3-7%). Diptera was the next group (2-6%), mainly due to the family Empididae (2-4%), and finally Dictyoptera (suborder Mantodea), with a negligible percentage (<1%).

Parasitoids

In both years, most of the parasitoids captured were immature stages belonging to order Hymenoptera (46-54% in 2009, 24-53% in 2010) that could not be determined to family level (Table S3 [suppl.]). They were either pupae or larvae found in parasitized hosts.

In 2009, adults belonging to eleven families of Hymenopteran parasitoids were captured, the most abundant specimens belonging to families Eulophidae (16-19%) and Scelionidae (13-16%). The next families in order of importance were Dryinidae (5-14%), Pteromalidae (1-5%) and Braconidae (0-4%). The remaining families appeared in percentages lower than 3%. In 2010, fourteen families were found. Again, Eulophidae (11-21%) and Scelionidae (10-16%) were the dominant groups. Dryinidae (0-14%), Encyrtidae (4-9%), Mymaridae (2-10%) and Figitidae (0-4%) were the next groups in the ranking of abundance, while the rest were found in percentages lower than 3%.

Other arthropods

The remaining arthropod community was very diverse and it was represented by more than forty families of eleven orders of classes Arachnida (infraclass Acari), Entognatha and Insecta (Table S4 [suppl.]). In both orchards, Thysanoptera was the most abundant group, but its percentage was much lower in 2009 (28-32%) compared to 2010 (80-91%), due to the very high number of thrips captured in 2010 (~1,400-1,800). This made also that the percentages of the other groups in 2010 were very low compared to the percentages obtained in 2009, though the number of specimens captured was more or less similar. Also in both years, the second group in order of importance was Coleoptera (22-23% in 2009, 4-15% in 2010). In 2009, the next most abundant groups were Hemiptera (9-14%), Diptera (9-12%), Psocoptera (6-11%) and Hymenoptera (3-6%). This ranking of abundance was slightly modified in 2010: Psocoptera (1-2%), Hemiptera (1-2%), Hymenoptera (0-3%) and Diptera (~1%). The remaining groups appeared in low percentages (~2 or lower in 2009 and <0.6% in 2010). Immature stages of different orders that could not be assigned to any family were classified as “Not identified” within the corresponding order. Also, a significant number of eggs were classified as “Not identified Arthropoda” because they could not be assigned to any taxonomic category (6-11% in 2009 and less than 2% in 2010).

Effect of kaolin and potassium salts of fatty acids combined with thyme essential oil (PSTEO) on non-target arthropods

Before the treatment applications both in 2009 and 2010, no significant differences were found between kaolin or PSTEO plots and untreated control plots in abundance of non-target arthropods except for the natural enemies in the PSTEO plots in 2009, which showed lower values compared to the control plots (Table 1). After treatment application, it was found a significant reduction in the kaolin-treated plots in the abundance of natural enemies in 2009 and 2010 and in the abundance of other arthropods in 2010 compared to the control plots (Table 1). PSTEO only produced a significant reduction in the number of natural enemies in 2010.

Concerning diversity and number of species of non-target arthropods, no significant differences were found between kaolin or PSTEO plots and the untreated control plots before treatment application both in 2009 and 2010 (Table 1). After treatment application, a significant reduction in the Shannon diversity index

and in the number of species was observed in the kaolin plots compared with the control plots in both years except for the Shannon index in 2010.

No differences in the community composition of non-target arthropods were found among treated and control plots before treatment application either in 2009 and 2010 (PRC analysis, $p > 0.05$) (Table 2). After treatment application, no effect was observed for both Kaolin and PSTEO-treated plots compared to the control ($p > 0.05$) in 2009, but a significant effect was observed in 2010 in the PSTEO-treated plots for the natural enemies community and in the kaolin-treated plots for the other non-target arthropod community (Table 2).

In those cases where the PRC analysis was significant, the contribution of different taxa to non-target arthropod community response in the treated plots is revealed by the species scores obtained. Taxa with a positive weight over 0.5 are expected to decrease in abundance compared to the control after treatment application. In the case of the effect of PSTEO on natural enemies in 2010, immature stages of parasitoids had the highest score (4.2), and the next most affected taxa were Anthocoridae (0.8) and Phytoseiidae (0.6).

Table 1. Abundance, Shannon index and number of species per sample of non-target arthropods (natural enemies and other arthropods) captured by beating in almond trees before and after being sprayed with kaolin or potassium soap with thyme essential oil (PSTEO) and in the untreated trees

Plots	Natural enemies ^a		Other arthropods ^a		Shannon index ^a		Number of species ^a	
	Before	After	Before	After	Before	After	Before	After
2009								
Control	5.6 ± 1.0	11.6 ± 1.6	7.6 ± 1.4	6.7 ± 1.5	1.7 ± 0.1	2.0 ± 0.1	7.9 ± 0.9	12.2 ± 1.5
Kaolin	6.9 ± 1.5	8.7 ± 1.6*	7.4 ± 1.6	5.0 ± 1.1	1.7 ± 0.1	1.8 ± 0.1*	8.1 ± 0.9	9.3 ± 1.3*
F	0.262	13.371	0.391	1.056	0.350	9.905	1.002	8.076
d.f.	1, 12	1, 11.9	1, 12	1, 12	1, 12	1, 11.2	1, 12	1, 27.1
p	0.618	0.003	0.544	0.324	0.565	0.009	0.337	0.008
Control	10.3 ± 1.7	7.6 ± 1.2	8.4 ± 1.3	5.1 ± 1.6	2.0 ± 0.1	1.8 ± 0.1	12.0 ± 1.4	8.2 ± 1.1
PSTEO	7.9 ± 1.4*	7.6 ± 1.0	7.6 ± 1.3	3.8 ± 0.9	1.9 ± 0.1	1.9 ± 0.1	10.3 ± 1.3	8.2 ± 0.8
F	6.532	0.003	1.847	0.112	3.231	0.678	3.454	0.159
d.f.	1, 9.3	1, 12	1, 12	1, 12	1, 24.2	1, 12	1, 12	1, 12
p	0.030	0.959	0.199	0.744	0.085	0.426	0.088	0.697
2010								
Control	3.7 ± 0.7	6.1 ± 0.5	77.6 ± 26.2	13.4 ± 3.0	1.1 ± 0.2	1.9 ± 0.1	6.1 ± 0.6	9.3 ± 0.7
Kaolin	5.0 ± 0.7	4.7* ± 0.7	92.3 ± 29.1	3.7 ± 0.6*	1.3 ± 0.2	1.6 ± 0.1	8.1 ± 0.6	5.9 ± 0.4*
PSTEO	5.1 ± 0.7	5.6* ± 0.7	68.8 ± 21.2	13.3 ± 3.5	1.2 ± 0.2	1.7 ± 0.1	7.3 ± 0.6	8.0 ± 0.7
F	1.598	5.489	2.787	11.761	2.387	2.265	2.683	6.317
d.f.	2, 17.8	2, 18	2, 18	2, 18	2, 18	2, 17.6	2, 60	2, 18
p	0.230	0.014	0.088	0.001	0.120	0.133	0.077	0.008

^a Values are mean per sample ± standard error. * indicates significant differences compared with the control ($p < 0.05$, linear mixed-effects model). Kaolin and PSTEO were compared with the control separately in 2009 because of the different time schedule of treatment application that year

Table 2. Significance of PRC analyses on the community of natural enemies and other non-target arthropods sampled by branch beating, before and after treatment application

Year	Group	Period	Comparison					
			Global		Kaolin vs control		PSTEO vs control	
			F ratio	p value	F ratio	p value	F ratio	p value
2009	Natural enemies	Before	–	–	1.140	0.7420	1.529	0.3960
		After	–	–	1.438	0.5420	1.017	0.8260
	Other arthropods	Before	–	–	1.189	0.5520	0.710	0.9080
		After	–	–	1.277	0.7300	1.094	0.6420
2010	Natural enemies	Before	1.987	0.3620	–	–	–	–
		After	3.691	0.0020*	2.070	0.0640	2.802	0.0060*
	Other arthropods	Before	3.129	0.1260	–	–	–	–
		After	6.393	0.0040*	5.309	0.0020*	1.427	0.6780

* indicates significant differences ($p < 0.05$, PRC analysis). The global comparison for the three treatments altogether could not be performed in 2009 because of the different time schedule of treatment application for kaolin and PSTEO. In 2010, the global comparison was first performed and when significant differences were found, separate comparisons between kaolin and potassium soap with thyme essential oil (PSTEO) vs the untreated control were subsequently performed.

For the effect of kaolin on other non-target arthropods in 2010, the affected taxa were (in decreasing order of effect) Melandryidae (3.3), Curculionidae (2.8), Formicidae (2.4), Psocoptera (2.0), Thysanoptera (1.4), Issidae (0.6), Phalacridae (0.6) and Anthicidae (0.5).

On the other hand, taxa with negative weights below -0.5 in the PRCs are expected to increase after treatment application. According to that, Theridiidae (-2.2), Philodromidae (-1.0), Coccinellidae (-0.7), Erythraeidae (-0.7) and Encyrtidae (-0.6) abundance increased after the PSTEO treatment and Tettigoniidae (-0.5) abundance increased after the kaolin treatment in 2010.

Discussion

One of the most important issues in integrated pest management strategies is the proper use of all the available methods to control pests, taking into account environmental concerns, like the possible negative effects of these methods on non-target fauna (Barzman *et al.*, 2015). In this respect, it is necessary to assess the influence of control strategies on those beneficial organisms that contribute to maintain populations of damaging arthropods under economic thresholds. This is especially important in organic production systems, because the availability of allowed products for pest control is more reduced than in conventional production.

There is few information about the composition of the natural enemies' community of almond orchards. Regarding predators, our results reasonably agree with those reported by Benhadi-Marín *et al.* (2011), who pointed out Araneae as the most abundant

group, followed by Coleoptera (mainly from Family Coccinellidae), Formicidae, Neuroptera, Hemiptera and Dermaptera. Other authors also report the occurrence of predators belonging to these groups in almond trees (Bolu, 2007; Eilers & Klein, 2009; Yanik *et al.*, 2011; Santos *et al.*, 2012). However, in none of these works the occurrence of predatory Acari, Thysanoptera or Diptera is reported, but Hoy *et al.* (1979) collected phytoseiid mites and predatory thrips in almond orchards, as well as Neuroptera and predatory Coleoptera. Of the families of spiders collected by Benhadi-Marín *et al.* (2011), Philodromidae, Salticidae, Theridiidae, Thomisidae, Araneidae, Oxyopidae and Gnaphosidae were also present in our orchards. Concerning parasitoids, in the work by Eilers & Klein (2009) the families Bethyidae and Encyrtidae comprised the majority of parasitoids collected, with only seven morphospecies captured, whereas we have collected up to 16 families of hymenopteran parasitoids. Regarding the possible natural enemies of *M. uncostata*, most of the groups reported by different authors (Vessia, 1961; Moleas, 1987) have been collected in our assays, and those that have been proved to feed on this lace bug in the field (Araneae and Anthocoridae) (Moleas, 1987) are well represented in both years of study.

In the search of more environmentally friendly strategies to control almond pests in organic production, some compounds like the aluminosilicate mineral kaolin, the insecticidal soaps based on potassium salts of fatty acids and plant essential oils have shown relatively good results in laboratory and field assays (Braham *et al.*, 2014; Sánchez-Ramos *et al.*, 2014; Marcotegui *et al.*, 2015). However, although these products are considered to have safe environmental

profiles (Weinzierl & Henn, 1991; Glenn *et al.*, 1999; Isman, 2006; Markó *et al.*, 2008; Regnault-Roger *et al.*, 2012), they cannot be considered as selective pesticides and their impact on non-target arthropod fauna should be evaluated.

In this work, we have observed some detrimental effects of kaolin and a combination of potassium salts of fatty acids and thyme essential oil on non-target arthropod fauna. Kaolin produced the most negative effects, since this product reduced the abundance of beneficials and other arthropods, and arthropod diversity and species richness in both years of the study. PSTEO only produced a slight decrease in abundance of natural enemies in the second year. Curiously, abundance of natural enemies in 2009 was significantly higher in control plots than in PSTEO plots before treatment application. This could be considered a random effect, since plots were assigned randomly to each treatment. In addition, no significant differences were observed after treatments.

Negative effects of kaolin on non-target arthropod fauna have been reported before in many crops, including olive, apple, plum, blueberry, pecan or cotton (Knight *et al.*, 2001; Showler & Sétamou, 2004; Lombardini *et al.*, 2005; Jaastad *et al.*, 2006; Markó *et al.*, 2006, 2008, 2010; Stelinski *et al.*, 2006; Sackett *et al.*, 2007; Pascual *et al.*, 2010; Scalercio *et al.*, 2010). These detrimental effects were independent of the number of applications made, because in these studies they ranged from one to more than ten. The affected taxa are very diverse and include a high number of families of Araneae (Salticidae, Philodromidae, Theridiidae), Acari (Phytoseiidae, Trombididae, Tydeidae), Coleoptera (Coccinellidae), Heteroptera (Anthocoridae, Miridae, Reduviidae), Dermaptera (Forficulidae), Neuroptera (Chrysopidae) or Hymenoptera (Formicidae and different families of parasitoids like Scelionidae, Pteromalidae, Aphelinidae, Braconidae, Ichneumonidae). In addition, different functional groups are affected, from predaceous and parasitoids to arthropods with other food habits. Laboratory studies have also shown some negative effects of kaolin on different beneficial arthropods like predatory Heteroptera (Anthocoridae), parasitoid Hymenoptera (Braconidae, Pteromalidae), Neuroptera (Chrysopidae) or Araneae (Bengochea *et al.*, 2013, 2014a,b; Benhadi-Marín *et al.*, 2016).

Regarding insecticidal soaps, some detrimental effects of different formulations have also been registered both in field and laboratory conditions on beneficial non-target arthropods belonging to Acari (Phytoseiidae), Coleoptera (Coccinellidae), Neuroptera (Chrysopidae) and Hymenoptera (Eulophidae, Braconidae) (Oetting & Latimer, 1995;

Smith & Krischik, 2000; Kraiss & Cullen, 2008; Raudonis *et al.*, 2010; Hall & Richardson, 2013; Smaili *et al.*, 2014). In addition, negative effects on predators and parasitoids have been reported for plant essential oils. Thus, predators belonging to Acari (Phytoseiidae), Coleoptera (Staphylinidae) and Heteroptera (Anthocoridae), and parasitoids belonging to Hymenoptera (Braconidae, Pteromalidae) showed some negative effects when treated with different essential oils from citronella, basil, soybean, rosemary, peppermint or *Chenopodium* (Momen & Amer, 1999; Amer & Momen, 2002; Choi *et al.*, 2004; Bostanian *et al.*, 2005; Huignard *et al.*, 2008; Cloyd *et al.*, 2009). However, the degree of magnitude of these effects can be considered much lower compared with the effect of kaolin, similarly to what has been obtained in our field assay with the combination of insecticidal soap and thyme essential oil.

With the aim of determining the most affected taxa after the treatment applications, PRC analyses were performed. However, the differences observed regarding abundance and diversity were not fully reflected in the PRC results, because significant effects were only detected in two analyses in 2010. This contrasts with former studies that indicated that PRC analyses were more sensitive than methods based on comparison of means (Pascual *et al.*, 2010).

As stated in our previous work, the intention was to evaluate the efficacy of the treatments with a reduced number of applications to establish cost-effective control strategies (Marcotegui *et al.*, 2015). These applications produced a moderate effect on almond tree pests and, though *M. unicastata* is one of the most affected taxa, a higher number of applications might be necessary to reduce damage effectively. However, we have found negative effects, especially with kaolin, on non-target arthropod fauna even with such low number of applications. Therefore, the increase in the frequency of applications would surely exert much higher disruption on the community of beneficial and other non-target arthropods.

In conclusion, despite the fact that the products assayed in the present work are claimed to be environmentally safer than those used in conventional agriculture, they still have detrimental effects on non-target arthropod fauna because of their reduced selectivity. This is especially relevant in the case of kaolin, because a continuous coverage of the plants throughout the season is essential for the effectiveness of this product, what might lead to long-term effects on the community of beneficial arthropods. Thus, a rational use of this type of products should be implemented, for example by alternating them with other strategies with less negative effects or by

taking into account the best timing for its application according to the phenology of pests.

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