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Side effects of different pesticides used in citrus on the adult stage of the parasitoid *Aphytis melinus* DeBach (Hymenoptera Aphelinidae) and its progeny

J. E. Gonzalez-Zamora*, M. L. Castillo and C. Avilla

Departamento de Ciencias Agroforestales. Universidad de Sevilla. Ctra. de Utrera, km 1. 41013 Sevilla, Spain

Abstract

Twelve pesticides commonly used in citrus in Spain were tested on adults of *Aphytis melinus* DeBach to determine their effects on parasitoid survival and fecundity, and the duration of the residue of each pesticide. Six of these pesticides were found to be harmless to moderately harmful to this parasitoid in a laboratory assay in closed Petri dishes: spinosad (bait formulation), azadirachtin, fenbutatin, fosetyl-Al, copper oxichloride, and mancozeb, with their scores on the reduction of beneficial capacity (RBC) index being between 21.4 and 94.6% after one week. The other six pesticides classified as harmful were tested on citrus plants to study their persistence over time under greenhouse conditions: Pirimicarb, pyriproxifen, paraffinic oil, abamectin, chlorpyrifos, and lambda-cyhalothrin. Most of these products reduced their negative effect on adults of *A. melinus* between one and six weeks after treatment, although lambda-cyhalothrin was still harmful to parasitoids 11 weeks after application. This information can help growers and consultants to make decisions about pesticide selection and application timing in citrus in order to support IPM implementation when *A. melinus* is present.

Additional key words: fecundity; IPM; mortality; parasitism; persistence.

Introduction

Integrated pest management (IPM) philosophy stresses the use as little pesticide as possible and only those compounds that are reasonably compatible with the natural enemies that are important in a particular crop. Moreover, in some parts of the world, such as the European Union, pesticide regulations have reduced the number of active ingredients available (*e.g.,* Council Directive 91/414/EEC; European Union, 2011). Determining the compatibility of pesticides with key biological control agents in a crop should be an ongoing activity, particularly in orchard crops such as citrus, where some key pests are under economically significant biological control (Kennett *et al*., 1999; Jacas & Urbaneja, 2010; Sorribas & García-Marí, 2010).

One of the key pests of citrus in Spain is the California red scale, *Aonidiella aurantii* Maskell (Hemiptera Diaspididae) (Franco *et al*., 2006; Jacas *et al*., 2010), and one of the most widespread and important natural enemies of this scale is *Aphytis melinus* DeBach (Hymenoptera Aphelinidae) (Kennett *et al*., 1999). This parasitoid, native to Pakistan and India, was introduced into California (USA) in the 1950s and since then in many citrus producing regions of the word (Kennet *et al*., 1999). In Spain it is present since the 1980s (Jacas *et al*., 2006) and it is well established in most citrus areas (Pina & Verdú, 2007; Sorribas *et al*., 2008).

Parasitoids of the California red scale can provide a good control of the pest if they are not killed or their activities impaired by pesticides (Grafton-Cardwell *et al*., 2008). However, classical biological control needs to be supplemented with inundative releases in cooler areas where they suffer a high overwintering mortality (Mazih, 2008; Zappalá *et al*., 2008; Grafton-Cardwell *et al*., 2011; Olivas *et al*., 2011). At present, *A. melinus* plays an important role in the control of the pest in many

* Corresponding author: zamora@us.es
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Abbreviations used: IOBC (International Organization for Biological Control); IPM (integrated pest management); PIEC (predicted initial environment concentration); RBC (reduction in beneficial capacity).

regions (Kennet *et al*., 1999) but in Spain neither him nor other parasitoids are able to keep *A. aurantii* populations below the damage threshold (Jacas *et al*., 2010).

Laboratory trials with pesticides are generally considered to be the first approach in order to detect their potential harm to beneficial species. Current protocols for detecting pesticides side effects, developed by organizations like the International Organization for Biological and Integrated Control of Noxious Animals and Plants (IOBC), recommend that products showing harmful effects in laboratory tests (run in the most negative conditions and with the most exposed stage of the beneficial species) should be further tested under more natural conditions, such as extended laboratory (to estimate residual life), semi-field, or field trials (Sterk *et al*., 1999). Under such conditions, harmful pesticides often are less detrimental to beneficial species than under laboratory ones.

Because of the importance of citrus as a crop, there are many studies that have determined the side effects of pesticides on parasitoids that are key natural enemies of citrus pests (Bellows & Morse, 1993; Bellows *et al*., 1993; Prabhaker *et al*., 2007, 2011; Suma *et al*., 2009; Vanaclocha *et al*., 2012). Continuous evaluation of new products allows the creation of data banks with the side effects of pesticides on natural enemies, as it happens with citrus (Jacas & Urbaneja, 2010; IVIA, 2012), which are of great importance to IPM implementation.

The active ingredients investigated in this study cover a wide range of products used in Spanish citrus, in both IPM and organic farming. We studied the side effects of these pesticides on *A. melinus*, first in laboratory trials with both fresh and aged residues, and afterwards in an extended laboratory trial to estimate the persistence and rate of degradation of the more harmful materials. The objective of this study was to provide information on the compatibility of pesticides and *A. melinus*, including the effect on progeny production, which can help to estimate potential effects on natural enemy populations. Information generated can be useful for farmers and consultants to take decisions on pesticide selection and application timing in citrus to support IPM implementation when *A. melinus* is present.

Material and methods

Tests followed, with adaptation, the principles of the IOBC/WPRS Working group "Pesticides and Beneficial Organisms" (Hassan *et al*., 1994; Sterk *et al*., 1999). Evaluations were done of (1) the contact toxicity to adult parasitoids after 24 h with fresh and 7-day-old residues, (2) duration of harmful activity (persistence) of residues of the more harmful products, and (3) effects on parasitization activity and sex ratio of parasitoid offspring.

Insect rearing

Adults of *A. melinus* used in the assays were reared in the facilities of the University of Sevilla, following the method developed by DeBach & White (1960) for rearing *Aphytis lignanensis* and later modified by others (Rose, 1990; Raciti *et al*., 2003). The procedure is based on first rearing the host (a parthenogenetic strain of *Aspidiotus nerii* Bouché) on butternut squash (*Cucurbita moschata* Duchesne ex Lamarck). When the host is in the second instar, the infested squash and adult parasitoids are placed together in a ventilated cage. Adult parasitoids emerge about 15 days later. Parasitoids produced in this manner were used in our experiments within 1 to 2 days of emergence.

Pesticides used in the assays

Twelve active ingredients were used in the assays, belonging to different groups and modes of action: organophosphates (chlorpyrifos), pyrethroids (lambda-cyhalothrin), insect growth regulators (pyriproxyfen), carbamates (pirimicarb), botanical insecticides (azadirachtin), microbiological insecticides (abamectin, spinosad as a bait to control *Ceratitis capitata* Wiedemann), organotins (fenbutatin), inorganic compounds (paraffinic oil, copper oxichloride), etylphosphonates (fosetyl-Al), and ditiocarbamates (mancozeb), the last three of these being fungicides. Formulations, field rates, concentrations used in the laboratory experiments, and target groups for each compound from citrus crops are given in Table 1.

Assay with fresh residues

This assay was carried out using residues on the inner walls of glass Petri dishes (5 cm diam.). Pesticides were applied to tops and bottoms of dishes at the laboratory concentrations (Table 1) with a potter preci-

Table 1. Pesticides tested for harmful effect on *Aphytis melinus* in citrus crop

Trade name, formulation and suppliers[a]	Active ingredient (%)	Field rate[b] (ppm a.i.)	Laboratory concentration[c] (ppm a.i.)	Targets
SunSpray®-LE, Agrichem, Madrid	Paraffinic oil 85 (w/v)	$12.8 \cdot 10^3$	$102.0 \cdot 10^3$	Mites, Scales
Vertan®-EC, Alcotan Lab., Sevilla	Abamectine 1.8 (w/v)	7.2	57.6	Mites, Lepidoptera
Aphox®-WG, Syngenta, Madrid	Pirimicarb 50 (w/w)	$5.0 \cdot 10^2$	$4.0 \cdot 10^3$	Aphids
Align®-EC, Sipcam Inagra, Valencia	Azadirachtin 3.2 (w/v)	24	$1.9 \cdot 10^2$	Aphids, Lepidoptera
Juvinal®10-EC, Kenogard, Barcelona	Pyriproxyfen 10 (w/v)	75	$6 \cdot 10^2$	Scales
Karate King®-WG, Aragonesas Agro, Madrid	Lambda-cyhalothrin 2.5 (w/w)	6.7	46.2	Aphids
Partner®-SC, Sipcam Inagra, Valencia	Fenbutatin 55 (w/v)	$5.5 \cdot 10^2$	$4.4 \cdot 10^3$	Mites
Closar® LE-EC, Sarabia, Lleida	Chlorpyrifos 48 (w/v)	$9.6 \cdot 10^2$	$7.7 \cdot 10^3$	Scales, Aphids, Lepidoptera
Spintor®cebo-CB, DowAgroSciences, Madrid	Spinosad 0.024(w/v)	0.12	0.96	Fruit flies
Ditiver®M-45-WP, Kenogard, Barcelona	Mancozeb 80 (w/w)	$3.2 \cdot 10^3$	$25.6 \cdot 10^3$	Diseases
Pombal®-WP, Sapec, Valencia	Fosetyl-Al 80 (w/w)	$2.4 \cdot 10^3$	$19.2 \cdot 10^3$	Diseases
Quimur®-WP, Sarabia, Lleida	Copper oxichloride 50 (w/w)	$1.0 \cdot 10^3$	$8.0 \cdot 10^3$	Diseases

[a] All suppliers are from Spain. [b] Field rates were calculated with the maximum label dose. a.i.: active ingredient. [c] Laboratory concentrations were obtained applying the PIEC calculation, using the field rate and a volume of 3,000 L of water ha^{-1}.

sion spray tower (Burkard Manufacturing Co. Ltd, Rickmansworth, Hertfordshire, UK), at 0.7 bar pressure. The tower was calibrated to leave 1.5 mg of solution cm^{-2}, and the concentration of the pesticides was calculated using the Predicted Initial Environment Concentration (PIEC) (Barret *et al*., 1994), with the expression: PIEC (μg cm^{-2}) = maximum field dose (g ha^{-1}) · f/100. This procedure left a residue on the glass surface that was equivalent in concentration to that left on leaves in field applications, considering a correction factor f depending of the type of crop (f = 1 for horticultural and arable crops, and f = 0.4 for trees).

Pesticides were left to dry at room temperature for an hour, and then 6 to 8 adult females of *A. melinus* that were 24-48 h old were introduced into each Petri dish, adding several drops of honey as food. Tops and bottoms of the Petri dishes were kept together using elastic bands. Petri dishes (4 to 6) were used for each pesticide as replications. Parasitoid mortality on each Petri dish was evaluated after 24 h. Surviving females of each Petri dish were transferred to a correspondent ventilated container with a piece of squash with drops of honey and an excess of *A. nerii* scales. Females were left with the scales for 48-72 h to parasitize the hosts offered, and the resulting parasitoid offspring were counted and sexed following their emergence. The experiment was carried out at 25 ± 1°C, a 16:8 L:D photoperiod and 60 ± 5% relative humidity.

Tests were run in four batches (designated as Experiments 1, 2, 3a, 3b) with each batch containing three-four pesticides (with the exception of 3b) and a control treated with distilled water. Test with fenbutatin was repeated in a separate experiment (3b) due to technical difficulties with the original trial.

Assay with 7-day-old residues

Eleven of the original 12 pesticides (fosetyl-Al was excluded from further study) were tested in a second assay, using 7-day-old residues. Materials were separated into two groups: harmful, when mortality in the fresh residues assay was 100%, and less harmful pesticides, when mortality in the fresh residues assay was lower than 100%. These two groups were run in separate experiments.

After products were sprayed on Petri dishes, these were left upside open, but covered with a filter paper, under room conditions (20 ± 2°C) for 7 days to allow residues to age and evaporate because the experimental units had no ventilation. After this period, 6 to 8 *A. melinus* females (24-48 h old) were introduced in each Petri dish, adding several drops of honey as food. Six Petri dishes were used for each pesticide and the control, as replications. Adult mortality and progeny production was evaluated as in the previous assay.

Persistence assay

The most toxic products found in the previous assays were selected to study their residue degradation over

time. This experiment was an extended laboratory test, where products were applied with a portable sprayer (Florabest, Abraham Diederichs GmBH & Co., Wuppertal-Germany) on young cv. Navelina orange trees, and the evaluation of residue persistence was made under laboratory conditions.

Three trees were sprayed with each pesticide at the maximum recommended field rate until run off at a pressure of 2 bars. Control trees were sprayed with tap water. The trees were kept in pots inside a greenhouse (located in the facilities of the Escuela Técnica Superior de Ingeniería Agronómica, University of Sevilla) for security reasons, irrigated every two days, and fertilized once a week throughout the course of the assay. The greenhouse was of 250 m^2, covered with a polyethylene plastic film of thickness 200 µm. The UVA radiation was measured inside and outside the greenhouse in several dates, with a reduction of 38.4 ± 2.6% of UVA radiation in the interior of the greenhouse. This experiment started at the middle of March 2011, with the application of the the pesticides, and lasted until the beginning of June 2011.

Toxicity of residues was evaluated 1, 2, 4, 6, 9, and 11 weeks after application of the pesticides. For this evaluation, three to four leaves were selected at random from the treated trees of each product and taken to the laboratory at the designated times. Three to four ventilated Petri dishes were prepared for each pesticide and the control at each occasion as replications, placing one or two leaves in each Petri dish over a moistened piece of filter paper. Ventilated Petri dishes were made by replacing most of the bottom half with a piece of organdy for ventilation, and this became the upper part of the test unit. Eight to 10 *A. melinus* females (24-48 h old) were introduced into each Petri dish, adding several drops of honey as food. Adult mortality and progeny production was evaluated as in the two previous assays.

Statistical analysis

Data on adult parasitoid mortality, the mean number of offspring produced per surviving parasitoid, and the sex ratio of the offspring (as % female) were subjected to one-way ANOVA using the results of each Petri dish as replicate. Means were separated using Tukey's honest significant difference test when analysis of variance were significant at $p < 0.05$. All data needing to be normalized were transformed before being analyzed. Percentages were subjected to arcsin square root (x) transformation, and the number of offspring to a log ($x + 1$) transformation. All analyses were performed using Statgraphics Centurion XVI (Stat Point Technologies, 2010). Levels of mortality were adjusted using the mortality level in the control and Abbott's formula (Abbott, 1925). An index "reduction of beneficial capacity (RBC)" was also calculated, as a parameter that integrates both mortality and offspring production, using the following formula (Overmeer & van Zon, 1982): $RBC\ (\%) = 100 - [(100 - M_c) \cdot R_t / R_c]$, where M_c = corrected mortality of the treated *A. melinus* females, R_t = reproductive performance of the treated *A. melinus* females, and R_c = reproductive performance of control *A. melinus* females.

Following the laboratory trials, pesticides were classified into four categories based on the levels of mortality and/or RBC: (1) harmless (< 30%); (2) slightly harmful (30-79%); (3) moderately harmful (80-99%); and (4) harmful (> 99%) (Sterk *et al*., 1999; Boller *et al*., 2005). For the persistence study in the extended laboratory method, pesticides were classified into four categories based on how long it took for mortality and/or RBC to reach a low value [evaluation categories, 1 = harmless (< 25%), 2 = slightly harmful (25-50%), 3 = moderately harmful (51-75%), 4 = harmful (> 75%)] or up to one month after treatment: A = short lived (< 5 days), B = slightly persistent (5-15 days), C = moderately persistent (16–30 days), and D = persistent (> 30 days) (Sterk *et al*., 1999).

Results

Assay with fresh residues

Assays with fresh residues (Experiments 1, 2, 3a, and 3b) caused the highest mortality, being 100% for pirimicarb and abamectin in Experiment 1 (Fig. 1a), for pyriproxyfen and chlorpyrifos in Experiment 2 (Fig. 1b), and for paraffinic oil and lambda-cyhalothrin in Experiment 3a (Fig. 1c). Some products tested produced a level of mortality no different from the control; these were azadirachtin and mancozeb in Experiment 1 (Fig. 1a), spinosad and copper oxichloride in Experiment 2 (Fig. 1b), and fosetyl-Al in Experiment 3a (Fig. 1c). Mortality for fenbutatin, in Experiment 3b, was greater than the control but not 100% (Fig. 1d).

Fecundity of treated wasps was similar to the controls for some products where adults survived, as for

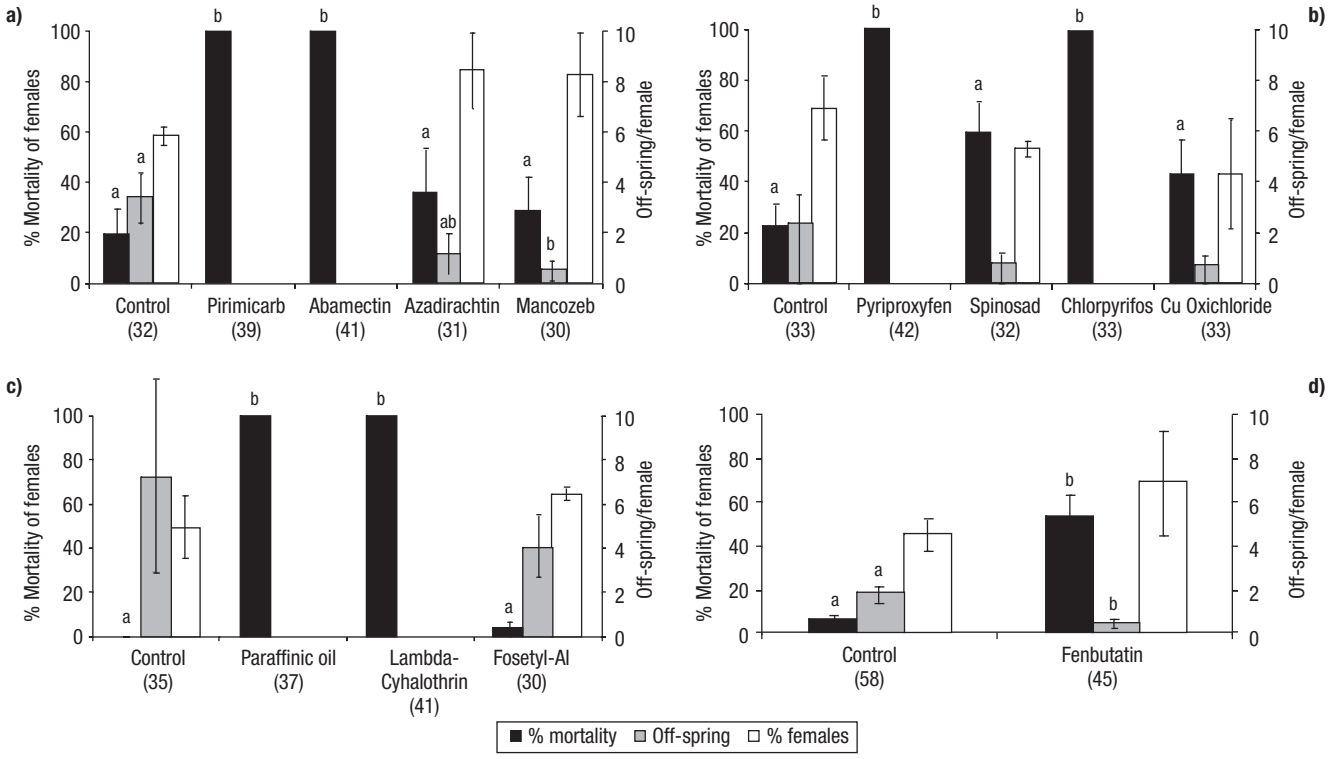

Figure 1. Mortality (%), offspring production, and percentage of female progeny in the different experiments with fresh residues of pesticides, using adult females of *Aphytis melinus*. a) Experiment 1, mortality ($F_{4,21} = 17.3$, $p < 0.0001$,), offspring production ($F_{2,11} = 4.61$, $p = 0.035$), % females progeny ($F_{2,6} = 2.15$, $p = 0.20$). b) Experiment 2, mortality ($F_{4,17} = 20.6$, $p < 0.0001$), offspring production ($F_{2,9} = 0.86$, $p = 0.45$), % females progeny ($F_{2,5} = 0.74$, $p = 0.52$). c) Experiment 3a, mortality ($F_{3,18} = 460.8$, $p < 0.0001$), offspring production ($F_{1,8} = 0.15$, $p = 0.71$), % females progeny ($F_{1,7} = 0.54$, $p = 0.49$). d) Experiment 3b (with only fenbutatin), mortality ($F_{1,22} = 23.9$, $p < 0.0001$), offspring production ($F_{1,12} = 6.25$, $p = 0.028$), % females progeny ($F_{1,9} = 1.38$, $p = 0.27$). Different letters above the same column represent statistical differences between means, using Tukey's HSD with $p = 0.05$. Columns without letters do not have statistical differences, with $p > 0.05$. Vertical bars represent the standard error. Numbers between brackets below the control and pesticide names represent the total number of females tested.

example azadirachtin in Experiment 1 (Fig. 1a), spinosad and copper oxichloride in Experiment 2 (Fig. 1b), and fosetyl-Al in Experiment 3a (Fig. 1c). Significantly reduced fecundity compared to the control was seen with mancozeb in Experiment 1 (Fig. 1a), and fenbutatin in Experiment 3b (Fig. 3d).

The sex ratio of the offspring of surviving parasitoids (as % female) was similar for all fresh residues of pesticides: Experiment 1 (Fig. 1a), Experiment 2 (Fig. 1b), Experiment 3a (Fig. 1c), and Experiment 3b (Fig. 1d). The overall proportion of females in this first assay was 61.9%.

Taking into account the RBC values of the fresh residues and the IOBC criteria, six products were classified as harmful, two as moderately harmful, and four as slightly harmful (Table 2). As fosetyl-Al produced very low mortality and had a reasonable low value of RBC (Table 2), it was excluded from further experiments with aged residues.

Assay with 7-day-old residues

For the assay with the 7-day-old residues, compounds were separated in two groups, based on their toxicity in the previous assay: Group 1, harmful products, and Group 2, moderately or slightly harmful products. Parasitoid mortality when exposed to 7-day-old residues of compounds in the Group 1 was 100% for all compounds (pirimicarb, paraffinic oil, abamectin, lambda-cyhalothrin, and chlorpyrifos) except pyriproxyfen (with 81.5 ± 11.9% mortality), all of which differed from the level of mortality in the control group (Fig. 2a). Parasitoids exposed to compounds of Group 1 (except for the control) neither survived nor produced offspring. Among offspring of the control group, 77.8% were female (Fig. 2a).

Parasitoid mortality did not differ among compounds and the control in the Group 2 (Fig. 2b). The number of offspring produced by parasitoids surviving

Table 2. Mortality of adults of *Aphytis melinus* (corrected with the Abbott's formula) and reduction of beneficial capacity (RBC) indices for 12 pesticides tested under laboratory conditions, with fresh and 7-day-old residues, using the laboratory concentrations calculated with the PIEC expression. IOBC classes of toxicity are refered to the RBC values obtained with fresh and 7-days old residues aged in the laboratory

	Fresh residues			7-day-old residues		
	Corrected mortality (%) (mean ± se)	RBC[a] (%) (mean ± se)	IOBC	Corrected mortality (%) (mean ± se)	RBC (%) (mean ± se)	IOBC
Paraffinic oil	100 ± 0	100 ± 0	4	100 ± 0	100 ± 0	4
Pirimicarb	100 ± 0	100 ± 0	4	100 ± 0	100 ± 0	4
Pyriproxyfen	100 ± 0	100 ± 0	4	76.8 ± 15.0	100 ± 0	4
Spinosad	47.8 ± 15.7	74.0 ± 21.6	2	13.3 ± 11.4	24.7 ± 9.9	1
Abamectin	100 ± 0	100 ± 0	4	100 ± 0	100 ± 0	4
Lambda-cyhalothrin	100 ± 0	100 ± 0	4	100 ± 0	100 ± 0	4
Chlorpyrifos	100 ± 0	100 ± 0	4	100 ± 0	100 ± 0	4
Azadirachtin	21.6 ± 21.0	77.7 ± 13.7	2	27.6 ± 18.0	65.7 ± 18.9	2
Fenbutatin	48.2 ± 11.7	93.0 ± 4.0	3	46.8 ± 19.1	58.6 ± 26.3	2
Fosetyl-Al	3.3 ± 3.3	31.7 ± 26.3	2	—	—	—
Mancozeb	10.0 ± 16.5	90.2 ± 6.4	3	36.7 ± 22.4	94.6 ± 3.4	3
Copper oxichloride	26.1 ± 18.9	66.0 ± 24.7	2	27.3 ± 19.7	21.4 ± 44.5	1

[a] Toxicity effects grouped into four categories according to IOBC: class 1, harmless (< 30%); class 2, slightly harmful (30-79%); class 3, moderately harmful (80-99%); class 4, harmful (> 99%).

exposure to products in Group 2 was lower for mancozeb compared with the control and spinosad (Fig. 2b), but there were no differences in the proportion of offspring that were female among the products (Fig. 2b), with the overall proportion of females being 60.3%.

The six products included in the harmful group (1) obtained the highest RBC values, and so were classified as harmful (Table 2). Based on their RBC values, two of the products in the other group (2) were classified as harmless, two as slightly harmful, and one as moderately harmful (Table 2).

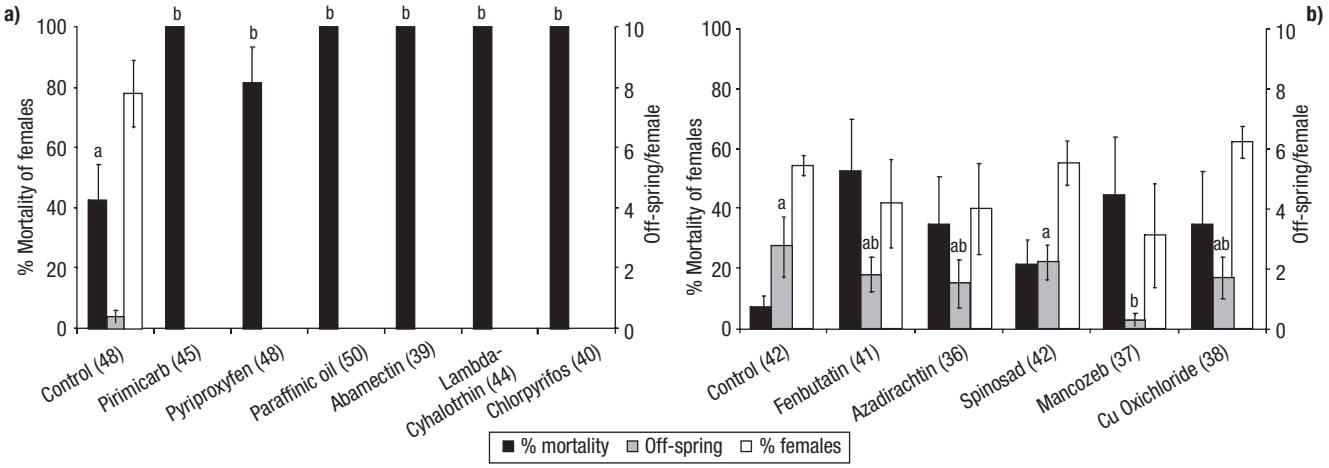

Figure 2. Mortality (%), offspring production, and percentage of female progeny in the two experiments with 7-day-old residues of pesticides, using adult females of *Aphytis melinus.* a) Experiment with harmful pesticides, mortality ($F_{6,38} = 15.5$, $p < 0.0001$). b) Experiment with moderately and slightly harmful pesticides, mortality ($F_{5,29} = 1.52$, $p = 0.21$), offspring production ($F_{5,23} = 2.67$, $p = 0.048$), % females progeny ($F_{5,21} = 1.20$, $p = 0.34$). Different letters above the same column represent statistical differences between means, using Tukey's HSD with $p = 0.05$. Columns without letters do not have statistical differences, with $p > 0.05$. Vertical bars represent the standard error. Numbers between brackets next to the control and pesticide names represent the total number of females tested.

Persistence assay

In the persistence assay, parasitoid mortality for most products was very similar to the control over the period of study, but in each week one or two products differed significantly from the control (Fig. 3a). The most toxic compounds were lambda-cyhalothrin, which caused 100% mortality from weeks 1-11, and chlorpyrifos, which caused mortality higher than the control in weeks 2 and 4.

The per capita production of offspring by surviving parasitoids was very similar among those products where some adults did survive as compared with the control (Fig. 3b) in week 1, week 2, and week 4. In week 6 there were a general decline in offspring production (Fig. 3b), with differences between products.

The proportion of parasitoid progeny that were female varied among products in some weeks of the study (Fig. 3c), as in week 1 (for pyriproxyfen and chlorpyrifos) and week 4 (for chlorpiryfos), but not in week 2 or week 6. Overall, the proportion of progeny that were female was 57.8%.

Mortality (corrected with Abbot's formula) in the persistence assay decreased over time, and even in the first week after pesticide application, parasitoid mortality was low for residues of most of the products with the exception of lambda-cyhalothrin, which caused 100% mortality through week 11, and chlorpyrifos, which caused a high mortality in weeks 1 to 4 (Fig. 4a).

RBC values decreased for most products until week 4, as could be expected, but in week 6 the values increased (Fig. 4b) due a general reduction in the off-spring

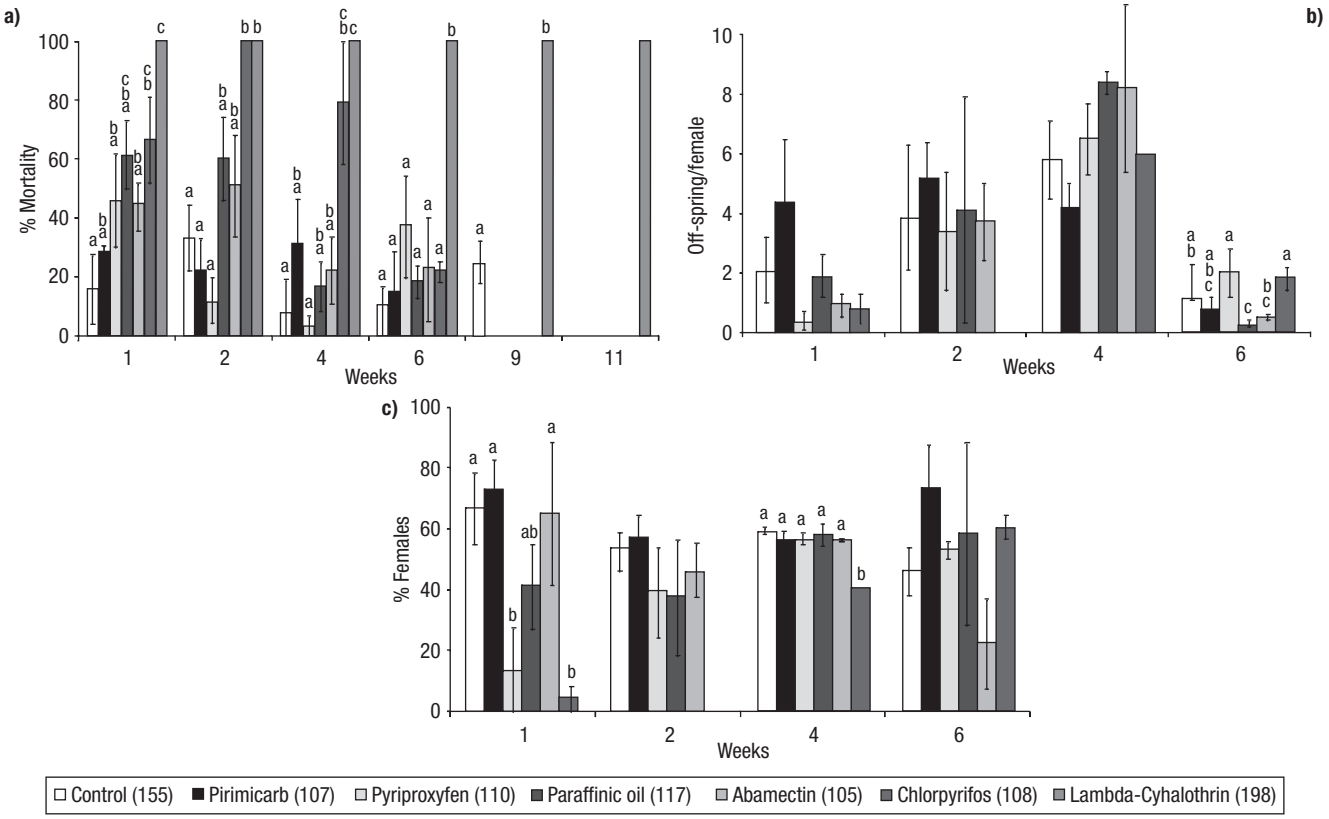

Figure 3. Evaluation of persistence of the most harmful products over time in an extended laboratory assay using pesticide-treated leaves on live, young orange trees. a) Mortality (%) of *Aphytis melinus* females (week 1: $F_{6,21} = 7.5$, $p = 0.0002$; week 2: $F_{6,21} = 10.5$, $p < 0.0001$; week 4: $F_{6,14} = 8.1$, $p = 0.0007$; week 6: $F_{6,14} = 8.1$, $p = 0.0006$; week 9: $F_{1,4} = 150.6$, $p = 0.0003$). b) Offspring production per *A. melinus* female (week 1: $F_{5,17} = 8.1$, $p = 0.31$; week 2: $F_{4,13} = 0.48$, $p = 0.75$; week 4: $F_{5,10} = 0.95$, $p = 0.49$; week 6: $F_{5,12} = 3.8$, $p = 0.028$). c) % of female offspring of *A. melinus* (week 1: $F_{5,17} = 3.3$, $p = 0.029$; week 2: $F_{4,13} = 0.7$, $p = 0.62$; week 4: $F_{5,10} = 4.3$, $p = 0.025$; week 6: $F_{5,12} = 1.0$, $p = 0.45$). Different letters within the same week represent statistical differences between means, using Tukey's HSD with $p = 0.05$. Columns within a week without letters do not have statistical differences, with $p > 0.05$. Vertical bars represent the standard error. Numbers between brackets next to the control and pesticide names represent the total number of females tested.

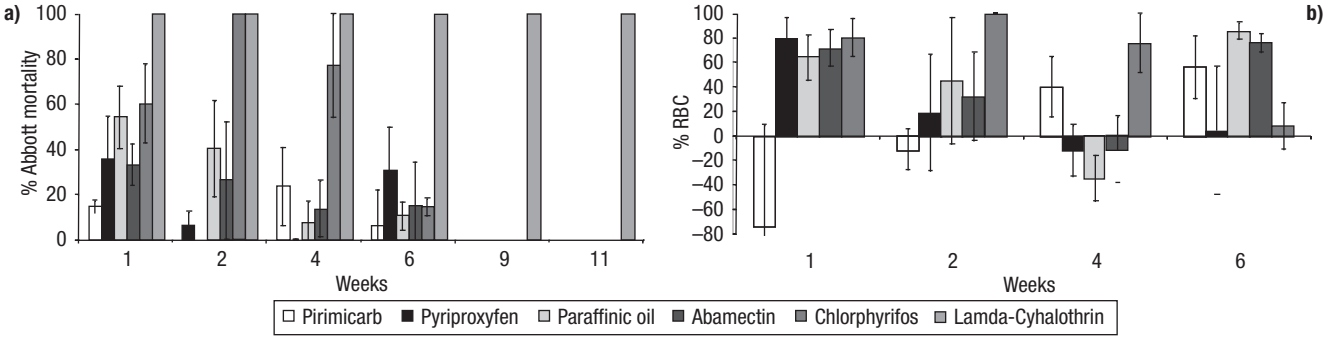

Figure 4. Toxicity of aged pesticides of several compounds to *Aphytis melinus* females in an extended laboratory assay using residues applied to young orange trees, where (a) is the corrected mortality using Abbott's formula and (b) is the reduction of beneficial capacity (RBC) index. Vertical bars represent the standard error.

production. The exception is pirimicarb, which showed higher offspring production than the control at the beginning of the assay (Fig. 3b), although not significantly, and for that reason its RBC values were negative.

Pirimicarb and pyriproxyfen were considered slightly persistent, as their corrected mortality rates and RBC values became quite low within 1-2 weeks after treatment (Table 3). Paraffinic oil and abamectin were moderately persistent, whereas chlorpyrifos and lambda-cyhalothrin were clearly persistent. This is most evident in the case of lambda-cyhalothrin, which caused 100% mortality through week 11 (Table 3).

Discussion

The implementation of biological pest control in citrus in Spain is increasing. However, the use of different types of pesticides is still necessary, making it important to study the side effects of such pesticides on the most prevalent natural enemies in citrus (Jacas *et al.*, 2010).

Five (spinosad, azadirachtin, fenbutatin, fosetyl-Al, and copper oxichloride) out of twelve pesticides tested caused little harm to *A. melinus* in experiments with fresh or 7-day-old residues in the closed Petri dishes, so they were excluded of the extended laboratory assay. Toxicity of these compounds was low seven days after application, so it is reasonable to expect that their persistence in real applications must be even lower. The exception is mancozeb, which although caused only low mortality, greatly reduced progeny production by surviving parasitoids (resulting in a high RBC value) in the laboratory experiments. For this reason it should be necessary to study its effect in a longer term and with more real conditions.

Table 3. Mortality of adults of *Aphytis melinus* (corrected with the Abbott's formula) and reduction of beneficial capacity (RBC) indices for six pesticides tested under extended laboratory conditions using young orange trees. IOBC classes of toxicity and persistence are refered to the RBC values in the table and the time to reach such values

	Corrected mortality (%) (mean ± se)	RBC (mean ± se)	Time[a] (weeks)	IOBC	
				Toxicity[b]	Persistence[c]
Pirimicarb	14.8 ± 2.7	–73.3 ± 82.7	1	1	(A)-B
Pyriproxyfen	0.1 ± 0.1	19.0 ± 47.5	2	1	B
Paraffinic oil	40.3 ± 21.3	45.2 ± 51.5	2 to 4	2 to 1	C
Abamectin	26.5 ± 25.9	32.1 ± 36.0	2 to 4	2 to 1	C
Chlorpyrifos	14.6 ± 3.9	8.3 ± 19.3	6	1	D
Lambda-Cyhalothrin	100 ± 0	100 ± 0	11	4	D

Residues were aged in a greenhouse. [a] Time to reach the values of corrected mortality and RBC showed in the table. [b] Toxicity effects grouped into four categories according to IOBC: 1, harmless (<2 5%); 2, slightly harmful (25-50%); 3, moderately harmful (51-75%); 4, harmful (> 75%). [c] Persistence, with four evaluation categories according to IOBC: A, short lived (< 5 days); B, slightly persistent (5-15 days); C, moderately persistent (16-30 days); D, persistent (> 30 days).

The products that were most harmful when presented as fresh residues (paraffinic oil, pirimicarb, pyriproxyfen, abamectin, lambda-cyhalothrin, and chlorpyrifos) were still harmful when presented as 7-day-old residues. Pirimicarb and pyriproxyfen are generally considered compatible with *A. melinus* in laboratory studies (Prabhaker *et al*., 2007; Rill *et al*., 2008; Zappalà *et al*., 2011), but our results showed harmful effects in closed Petri dish assays, even after seven days of aging and evaporation. Our results were obtained in the worst possible conditions for the insects. Pesticides concentrations used in the laboratory experiments were calculated to leave a pesticide deposit per surface unit similar to the used in field applications in citrus, which is a practice that few researchers follow in their works. Besides, experimental units were closed Petri dishes, and although a period of seven days was used to allow degradation and evaporation of components in the formulations, the results indicate that after this period pyriproxyfen (and other products considered compatible with *A. melinus*) was still harmful, if not completely in the mortality effect, at least in the overall evaluation (RBC) because no progeny was obtained from the surviving females.

Paraffinic oil was found to be harmful to *A. melinus* adults in our laboratory tests with closed Petri dishes. Also, when applied to larvae or pupae of several California red scale parasitoids, paraffinic oil caused very high mortality (Domínguez *et al*., 2003). Narrow-range mineral oil has also been found to be harmful to *A. melinus* (Zappalà *et al*., 2011). The other two products in the most toxic group, abamectin and chlorpyrifos, have been generally considered to be toxic in laboratory studies (but with low persistence) to *A. melinus* and other parasitoids found on citrus crops (Prabhaker *et al*., 2007; Campos *et al*., 2008; Suma *et al*., 2009), as they were here. We have not found studies on the effects of lambda-cyhalothrin on *A. melinus*.

The bait formulation of spinosad used in this work showed little harm to *A. melinus,* especially as 7-day-old residue, even though it was sprayed over the entire inside of the Petri dish. Its recomended use in field applications is spraying in localized spots. Although fresh residues of spinosad bait caused some mortality, it was not as high as reported by other works (Michaud, 2003). Spinosad baits (0.02% of active ingredient) showed no harm to *Aphytis* spp. or *Comperiella bifasciata* Howard populations in field trials (Thomas & Mangan, 2005), nor were harmful effects observed in laboratory trials for different natural enemies common in citrus orchards (Urbaneja *et al*., 2006). In another study, on the other hand, a more concentrated formulation of spinosad (44.2% of active ingredient) was harmful to *A. melinus* (Suma *et al*., 2009).

Most of the products (pirimicarb, pyriproxyfen, paraffinic oil, abamectin, and even chlorpyrifos) included in the persistence assay showed a decrease in their negative effects in the first week, under the more natural conditions (solar radiation, residues on leaf, dust) of this assay, in contrast to the effects of residues presented on glass in the laboratory (as in the first two assays). This was not true, however, for lambda-cyhalothrin, which remained highly toxic for at least 11 weeks.

Pirimicarb, pyriproxyfen, paraffinic oil, and abamectin can be considered slightly or moderately persistent, whereas chlorpyrifos and lambda-cyhalothrin were persistent. In spite of this, 6 weeks after treatment parasitoid mortality was very low for most compounds, with only lambda-cyhalothrin causing high mortality for a long period. These results agree with the recommendations of different insectaries regarding how long it is necessary to wait before releasing parasitoids after the application of these pesticides (Biobest, 2011; Koppert Biological Systems, 2011). The values of persistence of the compounds presented in this work must be considered, anyway, as maximum values, due the negative effect of the plastic cover used in the greenhouse on the compounds photodegradation. This is particularly right for abamectin, which is known to photodegradate very quickly due to UV radiation (Demchak & Dybas, 1997; Van de Veire *et al*., 2004), and whose residual effect on *A. melinus* in field conditions is very short (Morse *et al*., 1987). It is reasonable to expect that in normal outdoor conditions persistence values would be lower than the obtained in this work, but the practical consequence is that our persistence classification provide an extra security time for the parasitoids before their introduction in a particular grove.

Other compounds (as pyriproxyfen, mineral oil, and chlorpyrifos) have been also tested in field or semi-field conditions to assess their effects on natural enemies, being pyriproxyfen the most compatible with various natural enemies, including *A. melinus* (Grafton-Cardwell & Reagan, 1995; Grafton-Cardwell *et al*., 2006). Horticultural mineral oil (paraffinic oil) has also shown to be compatible in field conditions with a wide range of natural enemies found in citrus orchards (Liang *et al*., 2010).

Mortality rates seen in tests were consistent and generally had a decreasing slope over time (except for

lambda-cyhalothrin). In contrast, progeny production (included also within the RBC value) was more variable, especially in week 6, when there was a general decline in offspring production, which altered in some way the general trend.

Pirimicarb showed a different trend over time in its RBC value than other compounds. The negative values of this parameter for this compound mean that in some instances, progeny production of wasps exposed to this compound was actually higher than for the controls, although no significantly, as happened in weeks 1 and 2.

The work presented here provides information that growers and consultants can use to select pesticides compatible with *A. melinus* for use in a citrus IPM context. Information is presented not only on direct mortality of adults, but also on parasitoid progeny production, which is critical for the long term stability of the biological control of pests (Stark *et al*., 2007). Moreover, more information on pesticide residue persistence, as for example with mancozeb, is needed to help schedule inundative releases of *A. melinus* in citrus.

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