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The effect of emamectin benzoate on two parasitoids, *Aphidius colemani* Viereck (Hymenoptera: Braconidae) and *Eretmocerus mundus* Mercet (Hymenoptera: Aphelinidae), used in pepper greenhouses

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Abstract

Aphidius colemani Viereck and *Eretmocerus mundus* Mercet are two of the most representative parasitoids used as biological control agents (BCAs) primarily against aphids and whiteflies, respectively. The macrocyclic lactone emamectin benzoate (4”-deoxy-4”-methylamino-4”-epiavermectin B1 benzoate), an insecticide derived from the avermectin family of natural products, is being developed for controlling lepidopteran pests on a range of vegetable and other crops in Europe. The objective of this study was to determine the level of compatibility of the insecticide emamectin benzoate with *A. colemani* and *Er. mundus,* which are used in greenhouse vegetable production in southeastern Spain. Trials were conducted under commercial greenhouses to assess the effect of 1-, 3- and 7-day-old residues of emamectin benzoate at the highest recommended concentration (14.25 mg L^{-1}) on the population dynamics of the parasitoids. *A. colemani*, although released at higher rates than those commercially used, only reached low densities (less than 1 adult per plant), without significant differences among control plots and those treated with the compound. On the contrary, a natural infestation by native *Er. mundus* occurred and this trial can be considered as a direct-spray trial. Neither adult population nor parasitism on emamectin benzoate treated plots differed significantly from the control. Therefore, emamectin benzoate residues did not diminish *A. colemani* population densities if applied 1 day before the introduction of the arthropod (exposure to 1-day old residues). Similarly, emamectin benzoate was compatible with *Er. mundus* if a direct spray application was used on developed populations.

Additional key words: biological control; IPM; parasitism; residues.

Resumen

Efecto del insecticida benzoato de emamectina en dos parasitoides, *Aphidius colemani* Viereck (Hymenoptera: Braconidae) y *Eretmocerus mundus* Mercet (Hymenoptera: Aphelinidae) en invernaderos de pimiento

Aphidius colemani Viereck y *Eretmocerus mundus* Mercet son dos de los parasitoides más importantes que se utilizan como agentes de control biológico contra pulgones y moscas blancas, respectivamente. La lactona macrocíclica benzoato de emamectina (benzoato de 4”-deoxy-4”-(metilamino)-epiavermectina B1), insecticida derivado de la familia de las avermectinas, se está desarrollando para controlar plagas de lepidópteros en varios cultivos en toda Europa, incluyendo los hortícolas. En este estudio se determina la compatibilidad del insecticida con *A. colemani* y *Er. mundus*, utilizados en la producción hortícola del sureste español. Los ensayos se llevaron a cabo en invernaderos comerciales para estudiar los efectos de residuos de 1, 3 y 7 días de edad del insectida a la máxima concentración recomendada (14,25 mg L^{-1}) en la dinámica poblacional de los parasitoides. *A. colemani*, aunque liberado a dosis mayores de las

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Abbreviations used: AD (application date); BCA (biological control agent); DAI (days after introduction); DBI (days before introduction); IPM (integrated pest management).

utilizadas comercialmente, apenas consiguió establecerse (menos de un adulto por planta), sin detectarse diferencias significativas entre el control y los residuos del compuesto. Por el contrario, se produjo una infestación natural de *Er. mundus* y, por tanto, el ensayo correspondiente se consideró como un ensayo de aplicación directa. Ni la población adulta del parasitoide ni el parasitismo fueron significativamente diferentes entre el control y los residuos del compuesto. En conclusión, el benzoato de emamectina no redujo las poblaciones de *A. colemani,* cuando se aplicó un día antes de la introducción del insecto (residuo de un día). Igualmente, el compuesto fue compatible con *Er. mundus,* incluso en pulverización directa sobre una población ya establecida del insecto.

Palabras clave adicionales: control biológico; MIP; parasitismo; residuos.

Introduction

Tomatoes (*Solanum lycopersicum* L.) and sweet peppers (*Capsicum annuum* L.) are the two most important protected vegetable crops in the southeastern regions of Spain, Almería and Murcia (Urbaneja *et al.,* 2007).

Bemisia tabaci (Gennadius) (Hemiptera: Aleyrodidae), a key pest of many crops throughout subtropical and tropical regions of the world, causes also significant problems in protected agricultural systems in temperate regions (Naranjo, 2001). The impact of direct feeding and honeydew excreta that favors sooty mold production are factors that affect crop yield in both quantitative and qualitative terms (Oliveira *et al.,* 2001). However, the most economically significant losses are due to virus transmission, especially in tomatoes (Robledo-Camacho *et al.,* 2009). Chemical management is costly and, at best, provides only partial control because of the rapid development of resistance, a worldwide problem (Cahill *et al.,* 1996a,b; Viñuela, 1998; Kumar *et al.,* 2008). The sweet potato whitefly is currently managed through integrated pest management (IPM) programmes based on the release of commercial biological control agents (BCAs). The most successfully parasitoids used worldwide against *B. tabaci* belong to the aphelenid hymenopteran genera, *Encarsia* and *Eretmocerus*. *Eretmocerus mundus* Mercet (Hymenoptera: Aphelinidae) is effective against *B. tabaci* in the Mediterranean area (Calvo & Belda, 2006; Van der Blom, 2008; Van der Blom *et al.,* 2008), where natural populations are abundant in insecticide-treated and untreated vegetable and ornamental crops (Stansly *et al.,* 2005).

Other pests of lesser importance, such as green peach aphid *Myzus persicae* Sulzer (Hemiptera: Aphididae) and melon aphid *Aphis gossypii* Glover (Hemiptera: Aphididae), can be managed using inoculative introductions of *Aphidius colemani* Viereck (Hymenoptera: Braconidae) (Van der Blom, 2008). This solitary endoparasitoid has been reared on dozens of aphid species (Messing & Rabasse, 1995). It is commercially produced and widely distributed as an aphid biocontrol agent in glasshouses in several European countries.

However, because effective BCAs are not available for all pests, insecticides continue to be an important management tool in greenhouse IPM programmes. Among these pests, the lepidopterans, *Helicoverpa armigera* (Hübner), *Spodoptera exigua* (Hübner) and *Tuta absoluta* (Meyrick) are the primary targets for insecticide applications (Gabarra *et al.,* 2008; Van der Blom, 2008).

Emamectin benzoate (4”-deoxy-4”-methylamino-4”-epiavermectin B_1 benzoate) is a new insecticide developed to control lepidopteran pests on a wide range of crops worldwide, including vegetables (Liguori *et al.,* 2008). In Europe, the insecticide is under review for its inclusion in Annex I of the European directive 91/414/EEC, and it was recently approved for use on vegetables in Spain (MARM, 2012). The compound shows translaminar activity (Willis & McDowell, 1989), with rapid plant uptake and it is metabolised by photo-oxidation yielding non-toxic levels. This favours its selectivity for biological control agents (Ishaaya *et al.,* 2002). The effects of emamectin benzoate result from ingestion and to a certain extent from contact (Dybas & Babu, 1988). It suppresses the muscle contraction leading to eventual paralysis by stimulating the release of the neurotransmitter γ-aminobutyric acid (Ishaaya *et al.,* 2002). The insecticide is very active against *H. armigera*, *S. exigua* and *T. absoluta* (Liguori *et al.,* 2010; López *et al.,* 2010).

Because insecticides will likely remain a major component of pest suppression, minimising the effects of insecticides on BCAs is important in successful integration of biological and chemical control methods. Emamectin benzoate, registrated to control Lepidoptera, could be needed when different beneficials used to control other pests are present in the

crop. Among the natural enemies released in sweet peppers grown in southeastern greenhouses, we have chosen those controlling whiteflies and aphids. Previous laboratory studies indicated certain levels of harmfulness of topical application and fresh residues in related hymenopteran species, such as *Aphidius gifuensis* Ashmead (Hymenoptera: Braconidae) (Kobori & Amano, 2004) and *Encarsia formosa* Gahan (Hymenoptera: Aphelinidae) (Van de Veire & Tirry, 2003), respectively. With this background, assuming similar toxicity for *A. colemani* and *Er. mundus*, greenhouse trials were conducted to investigate the re-entry interval.

Therefore, the objective of this study was to determine the level of compatibility of the insecticide emamectin benzoate with *A. colemani* and *Er. mundus,* which are used in greenhouse vegetable production in southeastern Spain.

Material and methods

Methodology

In field tests, the experimental and control compounds were tested under typical conditions in a commercial crop following good agricultural practices. EPPO (European Plant Protection Organisation) guideline PP1/151(2) was consulted for the general aspects of the methodology used to perform these trials (EPPO, 2004).

Location, conditions and experimental design of the trials

To evaluate BCA population dynamics two trials (one per insect) were conducted under commercial greenhouse conditions during 2007 in Torre Pacheco, Murcia, Spain, in multiple-tunnel greenhouses of three-extruded-layer Indasol® (Solplast, Murcia) plastic at *ex* CIFACITA S.L. facilities. The greenhouses were automated with Aco® Vision Clima 727 equipment (Hortimax Growing Solutions S.L., Almería, Spain).

Trials with *Er. mundus* and *A. colemani* were performed to assess the effect of 1-, 3-, and 7-day-old residues on the population dynamics of the parasitoids. The trials started on 3rd August 2007, the transplantation date, and were completed on 20th December 2007, the date of the last evaluation. The trials were conducted under the following environmental conditions: 15-28°C, 64-93% (Temperature and relative humidity daily averages). In both trials, a fully randomised design with 3 replicates and 160 m^2 (272 plants) plots was used. To avoid arthropod cross-contamination and spray drift, the plots were isolated on both sides and on the top, with an anti-thrips net of 10 × 14 threads cm^{-2} (Botanica Equipment S.L., Alcantarilla, Murcia) sealed between the sides (anchored to the ground) and the top. This arrangement formed cages of 8 m width, 20 m length and 3.5 m heigth, separated and surrounded by 1 m corridors. The entrance to the plots consisted of overlaps of the lateral net (1.5-m overlap) closed by several wire loops.

Natural enemies

The parasitoids were supplied by Syngenta Agro S.A. Bioline (Aguadulce, Almería). The inoculations were done at the following rates: *Er. mundus* (Eretline® M Blisters) at 10 adults $plant^{-1}$, and *A. colemani* (Aphiline®) at 1.8 adults $plant^{-1}$. These release rates were higher than the commercially recommended rates (8-12 and 1-2 parasitoids m^{-2} respectively) to ensure a more rapid development of the parasitoid populations. The inoculative introductions were performed on 5th October, 1, 3 and 7 d after insecticides had been applied (1-, 3- and 7-day-old residues, respectively). *Er. mundus* was released by hanging a previously opened blister (each containing 150 parasitised hosts) on every 15 plants (a total of 18 blisters per plot) in the petiole of a middle leaf. For *A. colemani*, a bottle of 500 units (mummies and adults) was distributed as uniformly as possible through the plot with an application from the bottle every 5 plants in the growth area.

The viability and distribution of the population of BCAs was ensured by visually checking the existence of host populations prior the experiment or artificially infesting when needed. As such, the source of *B. tabaci* was a natural infestation in the *Er. mundus* trial, and the *M. persicae* originated from a previous artificial inoculation in the *A. colemani* trial. An artificial inoculation with *M. persicae* in the *A. colemani* trial was made on 10th September 2007 with infected shoots collected from a nearby pepper greenhouse in the same location. Fifteen infected shoots with ca. 10-30 aphids each were placed per plot. The shoots were uniformly distributed within the plot (approximately 1 infected shoot for every 18 plants).

Crop

The trials were conducted on pepper (*Capsicum annuum* L.) cv. Bilbo. The crop was cultured on artificial medium (Hidrosac® coconut fibre) (Poliexmur, S.A., San Ginés, Murcia), in a frame with 1.5 m row distance × 0.4 m plant distance (1.7 plants m^{-2}). The plants were transplanted on 3rd August 2007.

Insecticides

The formulated insecticide emamectin benzoate 95 g kg^{-1} SG (Affirm®, Syngenta Agro S.A., Madrid) was tested at the maximum recommended field concentration of active ingredient, 14.25 mg L^{-1}. The pyrethroid lambda-cyhalothrin (100 g L^{-1} SC) (Karate® Zeon®, Syngenta Agro S.A., Madrid) was also included as reference product, and applied at the recommended concentration of 20 mg L^{-1} of active ingredient, based on the known highly toxic effect of pyrethroids on most parasitoids and specifically demonstrated with bifenthrin on *Er. mundus* (Jones *et al.,* 1995), with bifenthrin and cyfluthrin on *Er. eremicus* Rose and Zolnerowich by Prabhaker *et al.* (2007) and with permethrin on *A. gifuensis* Ashmead by Kobori & Amano (2004).

Insecticides were applied as a foliar spray until runoff was observed. The application was performed with a motorised backpack sprayer Maruyama MS068 (Maruyama US. Inc, USA) and double-cone nozzle (1.5-mm diameter) gun, using a spray volume of 600 L ha^{-1} and an output pressure of 8 atm. The control plots were sprayed with tap water under the same specified conditions.

The products were sprayed on 4th October, 2nd October and 28th September 2007. This dates corresponds to periods of 1, 3 and 7 days, respectively, before introducing the parasitoids on 5th October 2007.

Sampling

Samples were collected from the 10 upper leaves of 20 plants $plot^{-1}$ randomly selected in the middle of the plots. The number of adults and the level of parasitism were assessed and reported as the number of adults per plant and the percentage of parasitism of *B. tabaci* nymphs for *Er. mundus*, or the number of parasitised *M. persicae* individuals (mummies) per plant for *A. colemani*. The number of adults was directly counted on the plant leaves. For the evaluation of the parasitised insects, leaves were collected in plastic bags from each plot. The leaves were evaluated under light microscope for parasitisation symptoms and retained in plastic cages until the emergence of adults.

Samples were collected at 7 and 0 days before introduction (DBI), and at 7, 15, 22, 30 and 38 days after introduction (DAI). The first evaluation after the introduction of the parasitoids was performed at 7 days to allow the population to naturally settle in the untreated plots. The remaining evaluations were made at approximately 7-day intervals after the application.

Data analysis

Data per sampling date were subjected to an analysis of variance (ANOVA) using Statgraphics® Plus v. 5.0 (STSC, 1987). Mean differences were analysed with an LSD test at a significance level of $\alpha = 0.05$ after the assumptions of normally distributed data (Kolmogorov test) and/or homoscedasticity (Bartlett test) were confirmed. Schneider-Orelli's formula (Schneider-Orelli, 1947) was used to calculate corrected mortality in comparison to the untreated control. Corrected mortality was used to rank the insecticides as harmless (<25%), slightly harmful (25-50%), moderately harmful (51-75%) or harmful (>75%) according to the IOBC standards for semi-field and field trials (Hassan, 1985).

Results

Eretmocerus mundus

Adult population

In this trial, a natural infestation by native *Er. mundus* occurred and was stable before the insecticide applications. Although the insect was released according to the original plans, this trial can be considered as a direct-spray trial in terms of the results and conclusions because insecticide applications were performed in the presence of the parasitoid.

One-day residues of lambda-cyhalothrin caused a significant population reduction from 7 DAI (harmful) ($F_{4,10} = 17.60$, $p < 0.001$) until the last evaluation at

38 DAI (moderately harmful) ($F_{4,10} = 7.17$, $p = 0.005$). None of the emamectin benzoate treatments (1-, 3- and 7-day-old residues) differed significantly from the control plots in any of the assessments during the experiment (7, 15, 22, 30, and 38 DAI) (harmless) (Table 1).

Parasitism

Results were similar in regards to the percentage of parasitised *B. tabaci* nymphs. One-day residues of lambda-cyhalothrin caused a significant reduction compared to the control in parasitism of *B. tabaci* nymphs by *Er. mundus*. The outcomes were ranked from slightly harmful at 7 DAI ($F_{4,10} = 6.87$, $p = 0.006$) to harmful at 15 DAI ($F_{4,10} = 31.23$, $p < 0.001$), 22 DAI ($F_{4,10} = 24.43$, $p < 0.001$), 30 DAI ($F_{4,10} = 26.60$, $p < 0.001$) and 38 DAI ($F_{4,10} = 22.00$; $p < 0.001$). None of the emamectin benzoate treatments (1-, 3- 7-day-old residues) differed significantly from the control plots in any of the assessments (harmless) (Table 1).

Aphidius colemani

Adult population

The 1-day-old residue of the reference product, lambda cyhalothrin, caused significant population reductions, from moderately harmful at 7 DAI ($F_{4,10} = 4.01$, $p = 0.034$) to harmful at 15 DAI ($F_{4,10} = 7.11$, $p = 0.006$), 22 DAI ($F_{4,10} = 8.14$, $p = 0.004$), 30 DAI ($F_{4,10} = 6.73$, $p = 0.007$) and 38 DAI ($F_{4,10} = 8.09$, $p = 0.004$). None of the emamectin benzoate treatments (1-, 3- and 7-day-old residues) differed significantly from the control plots in any of the assessments (harmless) (Table 2).

Table 1. Average *Eretmocerus mundus* population density (number of adults per plant) and parasitism (% parasitized *Bemisia tabaci* nymphs) over the different sampling dates when the natural enemy was exposed to aged residues (1, 3 and 7 days old) of emamectin benzoate. Studies on greenhouse pepper

Treatment	Conc. (mg L^{-1})	Sampling date						
		7 DBI[a]	0 DBI[a]	7 DAI	15 DAI	22 DAI	30 DAI	38 DAI
Population density (adults *E. mundus*/plant)								
Control		0.53 (±0.07)a	1.50 (±0.40)a	3.13 (±0.27)a	2.03 (±0.20)a	1.67 (±0.30)a	1.93 (±0.21)a	2.27 (±0.18)a
Emamectin benzoate 1 d (AD: 04/10/2007)	14.25	0.50 (±0.06)a	1.43 (±0.09)a	4.00 (±0.42)a	2.03 (±0.12)a	1.67 (±0.22)a	1.27 (±0.30)a	2.07 (±0.24)a
Emamectin benzoate 3 d (AD: 02/10/2007)	14.25	0.43 (±0.03)a	1.37 (±0.20)a	3.43 (±0.37)a	1.70 (±0.15)a	2.03 (±0.45)a	1.43 (±0.26)a	2.07 (±0.26)a
Emamectin benzoate 7 d (AD: 28/09/2007)	14.25	0.53 (±0.12)a	1.33 (±0.18)a	3.70 (±0.47)a	1.57 (±0.20)a	1.70 (±0.15)a	1.60 (±0.15)a	2.10 (±0.12)a
Lambda-cyhalothrin 1 d (AD: 04/10/2007)	20.00	0.83 (±0.18)a	0.57 (±0.09)a	0.33 (±0.12)b	0.23 (±0.09)b	0.63 (±0.12)b	0.73 (±0.09)b	1.03 (±0.03)b
Parasitism (parasitized *B. tabaci* nymphs)								
Control		42.67 (±3.38)a	60.00 (±1.53)a	69.67 (±1.33)a	80.33 (±1.45)a	60.67 (±1.76)a	56.67 (±2.40)a	65.33 (±2.91)a
Emamectin benzoate 1 d (AD: 04/10/2007)	14.25	39.33 (±4.10)a	62.67 (±3.53)a	67.33 (±6.12)a	68.67 (±1.76)a	61.33 (±4.37)a	50.67 (±5.81)a	58.00 (±5.29)a
Emamectin benzoate 3 d (AD: 02/10/2007)	14.25	44.67 (±2.33)a	59.33 (±3.33)a	67.00 (±4.16)a	70.00 (±3.06)a	58.00 (±1.15)a	55.33 (±3.53)a	62.00 (±3.06)a
Emamectin benzoate 7 d (AD: 28/09/2007)	14.25	42.33 (±4.67)a	62.33 (±2.60)a	68.00 (±4.51)a	74.67 (±8.51)a	62.67 (±4.37)a	56.40 (±2.66)a	56.00 (±6.00)a
Lambda-cyhalothrin 1 d (AD: 04/10/2007)	20.00	41.33 (±2.33)a	59.33 (±6.64)a	44.33 (±2.33)b	21.67 (±1.45)b	21.00 (±4.73)b	10.00 (±4.16)b	13.33 (±4.67)b

Means (± SD) within columns followed by the same letter are not significantly different (ANOVA, LSD; $\alpha = 0.05$). DBI: days before introduction. [a] A natural contamination of native *Er. mundus* occurred and was stable before the applications. Although the insect release was done according to the original plans, this trial can be considered as a direct spray one in terms of results and conclusions, because insecticide applications were made in presence of the BCA. DAI: days after introduction. AD: application date.

Table 2. Average *Aphidius colemani* population density (number of adults per plant) over the different sampling dates when the natural enemy was exposed to aged residues (1, 3 and 7 days old) of emamectin benzoate. Studies on greenhouse pepper

Treatment	Conc. (mg L^{-1})	Sampling date					
		0 DBI	7 DAI	15 DAI	22 DAI	30 DAI	38 DAI
Population density (adults *A. colemani* per plant)							
Control		0.00	0.30 (±0.03)a	0.22 (±0.04)a	0.13 (±0.03)b	0.40 (±0.03)a	0.47 (±0.04)a
Emamectin benzoate 1 d (AD: 04/10/2007)	14.25	0.00	0.30 (±0.03)a	0.18 (±0.03)a	0.17 (±0.03)ab	0.30 (±0.06)a	0.45 (±0.08)a
Emamectin benzoate 3 d (AD: 02/10/2007)	14.25	0.00	0.32 (±0.06)a	0.25 (±0.03)a	0.17 (±0.02)ab	0.37 (±0.11)a	0.43 (±0.10)a
Emamectin benzoate 7 d (AD: 28/09/2007)	14.25	0.00	0.33 (±0.09)a	0.27 (±0.04)a	0.23 (±0.03)a	0.37 (±0.04)a	0.48 (±0.04)a
Lambda-cyhalothrin 1 d (AD: 04/10/2007)	20.00	0.00	0.08 (±0.02)b	0.03 (±0.02)b	0.02 (±0.02)c	0.02 (±0.02)b	0.05 (±0.03)b

Means (± SD) within columns followed by the same letter are not significantly different (ANOVA, LSD; $\alpha = 0.05$). DBI: days before introduction. DAI: days after introduction. AD: application date.

Parasitism

Percentages of parasitism were lower than 1% both in treatments and control, so data were neither included in tables nor considered in terms of discussion.

Discussion

One application of emamectin benzoate on *Er. mundus* and *A. colemani* in the commercial crop in the greenhouse caused no detrimental effects either to the adult population of both parasitoids or to the efficiency of the parasitism by *Er. mundus* on the host, *B. tabaci*.

To our knowledge, the only information on the effect of emamectin benzoate on *Er. mundus* was reported by Sugiyama *et al.* (2011), who studied the toxicity of several insecticides on three endoparasitoids of *B. tabaci*: *Er. mundus, Er. eremicus* Rose & Zolnerowich and *En. formosa* Gahan in laboratory. The dipping method was used on the pupae and the insecticide was harmful to *Er. mundus*. The pupal stage is the development stage preferred to be used in many laboratory studies, showing generally a low mortality. González-Zamora *et al.* (1996) found that only 3 out of 13 insecticides and acaricides tested by foliar application with the Potter Tower were included in the moderately harmful category under the IOBC classification. Jones *et al.* (1998) tested six insecticides on *Er. mundus* pupae and found that only one half of these insecticides killed more than 50% of the pupae. Further experiments on the same parasitoid with indoxacarb, an insecticide also used to control lepidopterans, showed the same trend (González-Zamora *et al.,* 2004). The observation that the insect develops inside the pupal case of the whitefly is cited as an explanation of the low mortality found in the pupal stage after treatment with insecticides having very different modes of action. Nevertheless, adults emerging from the pupal stage can be less efficient as parasitoids or can have a shorter lifespan due to sublethal effects not measured in the experiments. Concerning to their effects on adults, any data are available on their toxicity to *Er. mundus* in laboratory. Moreover, references are contradictory regarding the toxicity of emamectin benzoate on adults belonging to the order Hymenoptera. The insecticide was harmful to the adults of *En. formosa*, based on a topical application of 15 mg L^{-1} under laboratory conditions (Van de Veire & Tirry, 2003). In other studies, fresh residues (10 mg L^{-1}) of the insecticide were harmful to the *A. gifuensis* and *Cotesia plutellae* Kurd (Hymenoptera: Braconidae) adults but not harmful to *Dolichogenidea tasmanica* (Cameron) (Hymenoptera: Braconidae), *Diglyphus isaea* (Walker) (Hymenoptera: Eulophidae) and *Trichogramma brassicae* (Bezdenko) (Hymenoptera: Trichogrammatidae). In contrast, one-day old residues were not toxic to these species (Chuckwudebe *et al.,* 1997; Haseeb & Amano, 2002; Hewa-Kapuge *et al.,* 2003; Van de Veire & Tirry, 2003; Kobori & Amano, 2004; Ruberson & Roberts, 2004). As reviewed by Naranjo (2001), it is difficult to find a common pattern in the laboratory evaluations of the toxicity of selected insec-

ticides (two insect growth regulators, imidacloprid and bifenthrin, a representative fourth generation synthetic pyrethroids) on four species of *Eretmocerus,* including *Er. mundus*. In brief, the effects on whitefly parasitoids, including both lethal effects and reproductive performance, were stage and species specific. Moreover, although systemic applications are generally harmless, foliar applications could be highly toxic (Naranjo, 2001; Kumar *et al.,* 2008).

It is too early to draw conclusions about the physiological activity of emamectin benzoate on *E. mundus,* because an insufficient number of studies have been conducted and because no extrapolation should be made in the light of our previous discussion.

The results of our study may be compared with previous findings on the toxicity of abamectin, the first avermectin that has a broader spectrum than emamectin benzoate. Abamectin has been reported to be highly toxic to the *B. tabaci* ectoparasitoid *Eretmocerus warrae* Naumann & Schmidt. Less than 1% emergence from any of the three developmental stages was recorded if treated with 1-2 mL L^{-1} of a sprayed application on the top and bottom surfaces of the leaves until runoff (Kumar *et al.,* 2008). The mummies of *Er. mundus* treated with abamectin and emamectin benzoate in our laboratory (M. Fernandez, unpublished results) were contaminated with the insecticides at the moment of the adult emergence. It seems they contact the residue of insecticides when abandon the pupal case. The treatments reduced emergence by 75% and 36%, respectively, compared with the controls.

Our results can be explained by the differences between laboratory and field conditions. Under laboratory conditions, in the "worst case scenario", a toxic compound can cause direct mortality of the parasitoids. In contrast, field conditions can produce reduced population densities and activity without the more severe effects commonly detected in the laboratory when compared with field studies. In the last, adult insects may escape from exposure pesticide applications via increased mobility and enemy free-space; early stages and pupae are less exposed on the leaf underside. In fact, the natural presence of *Er. mundus* in commercial greenhouses of sweet pepper, melon and tomato treated routinely with pesticides has also been reported by other authors in southeastern Spain (Rodriguez-Rodriguez *et al.,* 1994; González-Zamora *et al.,* 1996). This parasitoid maintained a high level of parasitism after exposure of pesticides (González-Zamora *et al.,* 2004).

In our *A. colemani* trial, 1-, 3 and 7-day-old residues of emamectin benzoate were harmless to adults. However, any conclusion could be drawn on the parasitism due to its low percentage in every plot. Factors to take into account are the insufficient aphid infestation level or the inappropriate environmental conditions for meeting the parasitoid population needs to have a normal parasitization index. Laboratory studies on *A. gifuensis*, showed a harmful effect of emamectin benzoate (10 mg L^{-1}) on adult females after being in contact with a sprayed surface for 24 hours. The compound still showed high mortality at 7 days, but its toxicity subsequently declined rapidly to be negligible as a 14-day residue (Kobori & Amano, 2004). Pupae inside *M. persicae* mummies were also sprayed showing lower toxicity than those against the adult females. Moreover, young pupae (12-h-old) expressed greater sensibility than older pupae.

Strategies that focus on the ecological selectivity should be exploited as an alternative if physiological selectivity fails to be effective in the combined use of chemicals and natural enemies. These approaches include reduced rates of application, temporal and spatial changes in application methods and changes in formulation and delivery and use of less persistent insecticides (Croft, 1990; Naranjo, 2001). The low persistence may explain the selectivity of emamectin benzoate aged residues found in the trial with *A. colemani.* Undetectable residues of the insecticide have been reported after 24 hours (Prabhu *et al.,* 1991) due to the rapid degradation of the residues of emamectin benzoate on the plant surface (Ishaaya *et al.,* 2002). As discussed by Lopez *et al.* (2010), several factors may impact the effect of aged residues of emamectin benzoate under greenhouse conditions compared to laboratory conditions, being the most important the rapid photodegradation of the insecticide (Jansson & Dybas, 1998).

In conclusion, emamectin benzoate can be considered compatible with *E. mundu*s in a direct-spray application and with *A. colemani* populations if applied 1 day prior to the parasitoid release.

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