



# Lethal and sublethal effects of spiropidion (TINIVION® technology), the new ketoenol, on *Eretmocerus eremicus* (Hymenoptera: Aphelinidae) and *Nesidiocoris tenuis* (Hemiptera: Miridae)

Flor Budia · Beatriz Dader · Sinisa Jelovcan · Robert Senn ·  
Fermin Amor · Pilar Medina

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**Abstract** *Bemisia tabaci* Gennadius (Hemiptera: Aleyrodidae) is a worldwide whitefly pest of many agricultural systems, causing damage directly through feeding and indirectly through the transmission of plant viruses. Spiropidion (TINIVION® technology) is a new ketoenol marketed by Syngenta that belongs to the inhibitors of acetyl CoA carboxylase. It is presumed to show excellent activity against whiteflies, aphids, thrips, and mites in vegetables and specialty crops via foliar and/or soil applications while having a favourable toxicological and environmental profile. Given that there are numerous natural enemies controlling the whitefly, it is necessary to study the joint use of this new compound with beneficials. The toxicity of spiropidion in comparison with spirotetramat and imidacloprid for the biological control of whiteflies was evaluated on pupae (laboratory, topical application) and adults (laboratory and semifield test, residual application) of *Eretmocerus eremicus* Rose and Zolnerowich (Hymenoptera: Aphelinidae),

and on adults of *Nesidiocoris tenuis* Reuter (Hemiptera: Miridae) (semifield test, residual application). Spiropidion resulted a slightly more selective compound than its counterpart spirotetramat for both natural enemies. Adult emergence, mortality and parasitization of surviving adults after application of spiropidion on *E. eremicus* mummies were classified as 1–2 International Organization for Biological Control (IOBC) categories, and also when adults were exposed to a treated tomato plant, regardless of age-residue (fresh or 8 days). Nevertheless, parasitization was reduced in the semifield assay for both residues (2–3 IOBC category). Spiropidion was perfectly compatible with *N. tenuis* in semifield conditions, as adult mortality was classified as 1 IOBC category for both residues, and surviving adults produced similar offspring rates than water-mock control.

**Keywords** Compatibility · IOBC · Tetramic acid · Spirotetramat · Imidacloprid · Toxicity

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F. Budia · B. Dader · P. Medina (✉)  
Unidad de Protección de Cultivos, Escuela Técnica Superior de Ingeniería Agronómica, Alimentaria y de Biosistemas, Universidad Politécnica de Madrid, Madrid, Spain  
e-mail: pilar.medina@upm.es

S. Jelovcan · R. Senn  
Syngenta Crop Protection AG, Basel, Switzerland

F. Amor  
Syngenta España, Madrid, Spain

## Introduction

Piercing sucking pests significantly reduce crop yield and quality through their sap and cell feeding behaviours (Kumar & Rathor, 2020). Many of these species (aphids and whiteflies) cause serious economic losses in intense agronomic cropping systems where insecticide control options are limited and resistance is an extremely serious problem (Nauen et al., 2008);

Jeschke, 2020). On the other hand, the number of available chemical classes for their control continues to decrease due to regulatory restrictions, with particular emphasis within the European Union. In order to circumvent these problems, and besides biological control, there is a need to develop new products that can provide improved efficacy, selectivity and favorable environmental profiles (Muehlebach et al., 2020). Although the insecticide market is currently dominated by compounds targeting nerve and muscle functions, new chemistries addressing other modes of action have been introduced in recent years (Lueke et al., 2020; Nauen et al., 2019). According to Sparks and Nauen (2015), the family of tetronec/tetramic acid derivatives or cyclic ketoenols, belonging to group 23 of the Insecticide Resistance Action Committee (IRAC) mode of action classification, was launched in the market in 2003. Three commercial products are available, the two tetronec acid derivatives spirotetramat and spiromesifen, and the tetramic acid derivative spirotetramat (Nauen et al., 2008; Jeschke, 2020). Spiropidion is a new tetramic acid family member insecticide and acaricide marketed by Syngenta already registered in some countries in Latin American (Paraguay, 2021, Brazil, 2023) and Asia–Pacific regions (Philippines/Malaysia, 2022; India, 2024). The four compounds are lipid biosynthesis inhibitors targeting acetyl-CoA carboxylase (ACC), an enzyme known to catalyze the carboxylation of acetyl-CoA to malonyl-CoA using ATP as a source of energy in fatty acid biosynthesis (Nauen, 2005; Lümmen et al., 2014; Jeschke, 2020). Since spiropidion belongs to the inhibitors of acetyl CoA carboxylase, it disrupts fatty acid biosynthesis, and is presumed to show excellent activity against whiteflies, aphids, psyllids, mealybugs, thrips, and mites in vegetables and specialty crops via foliar and/or soil applications. It has a favourable toxicological and environmental profile, and it is safe to pollinators and other non-target organisms (Muehlebach et al., 2020, 2021; Umetsu & Shirai, 2020).

The cotton whitefly *Bemisia tabaci* Gennadius (Hemiptera: Aleyrodidae) is a worldwide species (Boykin et al., 2007) of many agricultural systems including various vegetable, ornamental and field crops (Byrne & Bellows, 1991; De Barro et al., 2011; Stansly & Naranjo, 2010), causing damage directly through feeding and indirectly through the transmission of plant pathogenic viruses, primarily

begomoviruses (Jones, 2003). This phloem-feeding insect is characterized by short life-cycle and high fecundity, which make it a species especially dangerous for agriculture as well as by its exceptional ability to develop resistance to insecticides (Byrne & Bellows, 1991). Of all whiteflies species, *B. tabaci* remains one of the most economically important pests of vegetable and ornamental crops worldwide (Sani et al., 2020; Stansly & Naranjo, 2010). The management of *B. tabaci* populations and the diseases it transmits is difficult, among others, by the quick development of resistance to major insecticide classes (Horowitz et al., 2011, 2020; Bielza et al., 2019). The use of insecticides remains the primary means of control, especially because it often causes severe outbreaks and damage in spite of its extensive enemy fauna (Horowitz et al., 2011; Arnó et al., 2010). However, due to resistance and environmental inconveniences, the consideration of alternative strategies is extremely important. Among those strategies that need to be examined is the use of augmentative biological control.

*Eretmocerus eremicus* (Rose and Zolnerowich) (Hymenoptera: Aphelinidae) is a parasitoid of the second and third larval instars of the cotton whitefly *B. tabaci* and of the greenhouse fly *Trialeurodes vaporariorum* Westwood (Hemiptera: Aleyrodidae), mostly in greenhouses of Mediterranean countries. It is considered a better parasitoid in comparison to the genus *Encarsia* (the other major taxon of aphelinids attacking whiteflies), because it is not known that *Eretmocerus* species exhibits autoparasitism: a potentially deleterious trait to biological control (Bellamy et al., 2004; Stansly & Naranjo, 2010; Sani et al., 2020). *Nesidiocoris tenuis* Reuter (Hemiptera: Miridae) is native and widely used as a Beneficial Control Agent (BCA) in the Mediterranean Basin to control several tomato whiteflies and *Tuta absoluta* Meyrick (Lepidoptera: Gelechiidae) (Urbaneja et al., 2013; Pérez-Hedo & Urbaneja, 2016; Bhatt & Patel, 2018; Konan et al., 2021). It is also known that it can damage crops due to its zoophytophagous habits. It causes necrotic rings on stems and leaf petioles and flower dropping due to its repeated feeding around stems and flowers, punctures on fruits and ultimately wilting in tomato (Sanchez, 2008); Calvo et al., 2009; De Puyseleir et al., 2013; Souto et al., 2022) often requiring the use of insecticide to reduce population density (Castañé et al., 2011). Despite all these

disadvantages, it is a key natural enemy on tomato crops due to its ability to maintain its population density even when the pest prey density is extremely low (Pérez-Hedo & Urbaneja, 2016; Shibata et al., 2024; Symondson et al., 2002).

One of the main premises in IPM systems is to use pesticides with proven selectiveness to biological control agents (BCAs). Therefore, the evaluation of the side effects of pesticides on useful fauna is critical for developing effective IPM strategies (Garzón et al., 2015). As a consequence, this work aimed at evaluating the compatibility of three different concentrations of spiropidion with the natural enemies *E. eremicus* and *N. tenuis* in laboratory and semifield tests.

## Materials and methods

### Insects

*Eretmocerus eremicus* (Ercal, cardboard with 100 mummies each) were purchased from Koppert (Almería, Spain) and used straightforward for experiments. *Nesidiocoris tenuis* (Nesibug, bottle with 500 adults each) came from Koppert (Almería, Spain). Experiments were carried out during spring in 2018. Once received, *N. tenuis* were maintained inside a ventilated methacrylate cage (40×30×30 cm), with 5 feeders (3.5 cm diameter) containing eggs (3.5 g) of *Ephestia kuehniella* (Zeller) (Lepidoptera: Pyralidae) (Entofood®, Koppert, Almería, Spain) and fresh green beans (*Phaseolus vulgaris* L., 500 g), previously washed with sodium hypochlorite at 1.15% (Amukina, Madrid, Spain), as sprue and oviposition substrate. In order to have a continuous rearing, beans with eggs were replaced three times a week and placed inside ventilated PVC cages (22×10×7 cm), with a feeder (1 cm diameter) containing *E. kuehniella* eggs (0.4 g). Once nymphs reached N3-N4 stage, they were moved to the methacrylate box previously described. The cycle lasted 23 days from egg to adult inside a walk-in climate chamber (4.25 m×2 m×2.5 m) with controlled conditions (27 °C, 60% RH, photoperiod 16L:8D).

### Plants

Tomato (*Solanum lycopersicum* L. cv. Marmande) and pepper (*Capsicum annuum* L. cv. Dulce italiano)

were sown in seedbeds containing 1:1 soil and vermiculite (Projar, Madrid, Spain) inside a greenhouse with controlled conditions (21.5±0.24 °C, 52.3±0.72%, natural photoperiod). Once seedlings reached 3 true leaves (solanaceous BBCH scale 13), they were transplanted. Pepper leaves were used for laboratory trials as tomato leaves lose quickly their turgor once they are detached from the plant. Semifield trials were performed on tomato.

### Insecticides

A novel active ingredient, spiropidion was supplied by Syngenta España and tested at 0.8, 1.2 and 1.6 ml L<sup>-1</sup> according to the manufacturer recommendations. Two synthetic active ingredients were included as standards for comparison: 1) Spirotetramat (Movento 100 SC, Bayer, Valencia, Spain), a systemic tetramic acid derivative, acting by ingestion against woodlice, psyllids, aphids and whiteflies and latest ketoenol in the market (same IRAC group as spiropidion), and 2) imidacloprid (Confidor 200 SL, Bayer, Valencia, Spain), a systemic neonicotinoid, competitive modulator of the nicotinic acetylcholine receptor, acting by ingestion against whiteflies, aphids, beetles and leaf suckers (positive control routinely used in our research group in toxicity trials) (IRAC, 2024). Cross-resistance was observed between spirotetramat and spiropidion. However, no significant cross-resistance was found between neonicotinoids and tetramic acid insecticides (Liu et al., 2025). Spirotetramat was used at 1.34 ml L<sup>-1</sup> and imidacloprid at 0.75 ml L<sup>-1</sup>. A water-mock negative control was also included in every experiment.

### Laboratory toxicity on *E. eremicus*

We studied adult emergence and progeny under laboratory conditions. Cardboard cards with *E. eremicus* mummies (60–90) were placed in glass Petri dishes and insecticides were applied topically with hand sprayers until run-off (Matabi Berry 1.5, Goizper S. Coop., Gipuzkoa, Spain) to ensure the complete treatment of the test cards and mimic a real application in the field. Once dry, each card was introduced in a ventilated PVC cage (7 cm diameter, 3 cm high) containing drops of honey as food. Cages were maintained inside a climate chamber (Sanyo MLR 351, Panasonic Group, Madrid, Spain) with controlled

conditions ( $22 \pm ^\circ\text{C}$ ,  $50 \pm 10\%$  RH, photoperiod 16L:8D). Adult emergence was staggered. Emerged individuals were separated daily for ten days, when no more adults emerged in the negative control. They were placed in the cages previously described, but with one untreated pepper leaf in order to reduce the static electricity in the plastic box. Mortality of adults was evaluated at 72 h following their emergence to comply with IOBC (International Organization of Biological Control) guidelines.

Surviving adults (when they accounted for a minimum of 50% of the total) were sexed using a magnifying glass. As many pairings of one male and one female as possible were formed. After 72 h, each pair was placed in a cage containing one pepper leaf infested with a known number of *B. tabaci* N2 nymphs, restricted to a circular area (3 cm diameter, mean  $10.13 \pm 1.34$  nymphs per day), and drops of honey as food. A new infested pepper leaf was offered daily during 5 consecutive days. Leaves with parasitized nymphs were moved to maintenance units consisting of a glass lower plate (10×5 cm), a wipe with a central hole (3.5 cm diameter), the leaf with the back facing up, and a plastic upper plate (10×5 cm) with a central hole (3.5 cm diameter). The unit was fastened. Daily, the wipe was saturated with water using a syringe to minimize interference. One week later, the number of parasitized nymphs per female was counted. Units were maintained inside the climate chamber described in the previous paragraph.

Semi-field toxicity of fresh residue and persistence on *E. eremicus* and *N. tenuis*

Insecticides were applied on tomato plants (BBCH scale 13) with hand sprayers until run-off to mimic a real application in the field. Fresh (1-day old residue) and persistence (8-day residue) were studied.

For *E. eremicus*, the experimental unit consisted of a pot (14 cm diameter) with one tomato plant, covered with a ventilated plastic cylinder (15 cm diameter, 20 cm high). White ethylvinyl acetate rubber (20×20 cm) was placed around the stem at soil level. The stem was secured with plasticine. A feeder (3 cm diameter, 3.5 cm high) containing 30% honey in water was placed on the rubber. Three cardboard cards with a known number of mummies were placed at different heights, just after the treatment. After 24 h, the cards were removed and mummies that had not emerged were

counted. After 72 h, adult mortality was evaluated. Surviving adults were sexed, paired and the parasitism was evaluated as described in the previous section.

For *N. tenuis*, the experimental unit consisted of a pot (22 cm diameter) with one tomato plant, covered with a ventilated plastic cylinder (22 cm diameter, 25 cm high). White rubber (25×25 cm) was placed around the stem at soil level. The stem was secured with plasticine. A feeder (3 cm diameter) containing *E. kuehniella* eggs (3.5 g) was placed on the rubber. Ten young adults (<72 h) were introduced. After 72 h, adult mortality was evaluated. Surviving adults were removed from the cage and sexed. Fifteen pairings of one male and one female were formed. Then, each pair was placed in a ventilated PVC cage (7 cm diameter, 3 cm high) containing a green bean piece and *E. kuehniella* eggs (0.4 g). The number of nymphs per female was counted for 6 days once they have reached N2. Experimental units were maintained inside the climate chamber described in the previous section.

## Statistics

Data are presented as mean±SEM, and were analysed using Statgraphics Centurion 19 version (The Plains, Virginia, USA). Parameters studied were compared by unifactorial analysis of variance ANOVA followed by LSD mean comparison test ( $p < 0.05$ ) after checking premises for parametric analysis (normality, homocedasticity). Adult mortality was corrected with Schneider-Orelli formula, and *E. eremicus* parasitization and number of *N. tenuis* nymphs with Abbot formula (Abbott, 1925; Püntener, 1981). The pesticide effect was categorized according to the four IOBC toxicity categories, based on mortality or reduction of the studied parameters (Hassan, 1994). For laboratory conditions, 1=harmless: <30%; 2=slightly harmful: 30–79%; 3=moderately harmful: 80–99%; or 4=harmful: >99%. For semi-field conditions, 1=harmless: <25%; 2=slightly harmful: 25–50%; 3=moderately harmful: 51–75%; or 4=harmful: >75%.

## Results

### Laboratory toxicity on *E. eremicus*

The application of spiropidion at  $1.6 \text{ ml L}^{-1}$  and spirotetramat on mummies returned significantly fewer

adult emergence than water-mock control (Table 1). According to the toxicological categories established by IOBC, spiropidion was innocuous and spirotetramat was slightly toxic. Emergence was nearly suppressed after treatment with the positive control imidacloprid; therefore, the percentage of parasitization could not be evaluated. Spirotetramat caused slightly higher adult mortality than water-mock, although not enough to be considered even moderately toxic according to IOBC categories (Table 1) since both spiropidion and spirotetramat were harmless for this parameter. After pairing the surviving adults from each treatment, the number of parasitized nymphs per female after treatment with both compounds, spiropidion and spirotetramat was lower in comparison to the controls, but not significantly different. Nevertheless, the reduction in numbers of parasitized nymphs was higher after spirotetramat treatment in comparison with spiropidion. Both ketoenols were classified as 1–2 according to IOBC categories (Table 1).

#### Semi-field toxicity of fresh residue and persistence on *E. eremicus* and *N. tenuis*

For *E. eremicus*, in the case of fresh residue (1-day-old), adult mortality was significantly highest after treatment with imidacloprid (Table 2). Spiropidion at 1.2 and 1.6 ml L<sup>-1</sup> and spirotetramat caused significantly more adult mortality than water-mock control. Nevertheless, both insecticides were classified as innocuous according to IOBC toxicological categories. The parasitization of surviving adults was low and similar in every treatment (Table 2). Both, spiropidion and spirotetramat were categorized as 2–3. In the case of persistent residue (8-day-old), every insecticide caused significantly more mortality than water-mock (Table 2). Spiropidion and spirotetramat were classified as slightly toxic. As happened in fresh residue, parasitization was low and statistically similar to the control. When comparing both residues at 0.8 ml L<sup>-1</sup> spiropidion, mortality was significantly higher at 8 days.

For *N. tenuis*, in the case of fresh residue (1-day-old), adult mortality was low and statistically similar in water-mock control, spiropidion and spirotetramat (Table 3). Mortality was nearly total after treatment with the positive control imidacloprid. Thus, spiropidion and spirotetramat were classified as innocuous, and imidacloprid as moderately toxic according to

**Table 1** Adult emergence, adult mortality and parasitization of surviving adults after topical application of different insecticides (spiropidion, spirotetramat, imidacloprid) on *Eremocerus eremicus* mummies placed in ventilated cages under laboratory conditions

Treatment	Dose (ml L <sup>-1</sup> )	Emergence		Mortality at 72 h		Parasitized nymphs per female				
		%	IOBC <sup>a</sup> Replicates	%	IOBC Replicates	Number	IOBC Replicates			
Water-mock		54.05 ± 6.22 c	-	5 cards containing 60–90 mummies each	9.68 ± 3.67	-	5 cards containing 60–90 mummies each	4.17 ± 2.15	-	6 females
Spiropidion	0.8	42.92 ± 4.39 ab	1	60–90 mummies each	6.95 ± 2.63	1	60–90 mummies each	2.20 ± 0.97	2	5 females
	1.2	46.87 ± 2.05 bc	1		11.59 ± 4.16	1		3.33 ± 2.85	1	5 females
	1.6	39.22 ± 2.80 b	1		12.30 ± 2.08	1		2.25 ± 1.11	2	5 females
Spirotetramat	1.34	34.84 ± 2.80 b	2		21.23 ± 4.56	1		1.20 ± 0.49	2	5 females
Imidacloprid	0.75	1.16 ± 0.80 a	3		-	-		-	-	-
Statistics		$F_{5,24} = 18.77; P < 0.001$			$F_{4,20} = 2.30; P = 0.094$			$H = 0.52; P = 0.97$		

Lowercase letters within the same column stand for significant differences among treatments according to unifactorial ANOVA or Kruskal–Wallis ( $P < 0.05$ )

<sup>a</sup>International Organization for Biological Control toxicity categories for laboratory conditions, based on mortality or reduction of the parameter: 1 = harmless: <30%; 2 = slightly harmful: 30–79%; 3 = moderately harmful: 80–99%; or 4 = harmful: >99%

**Table 2** *Eretmocerus eremicus* adult mortality and parasitization of surviving adults after run-off treatment of different insecticides (spiropidion, spirotetramat, imidacloprid) on tomato plants under semi-field conditions

Treatment	Dose (ml L <sup>-1</sup> )	Fresh (1-day residue)				Persistence (8-day residue)							
		Mortality at 72 h		Parasitized nymphs per female		Mortality at 72 h		Parasitized nymphs per female					
		%	IOBC <sup>a</sup>	Replicates	Number	IOBC	Replicates	Number	IOBC	Replicates			
Water-mock		10.01 ± 3.74 a	-	10 plants	1.44 ± 0.38	-	9 females	12.00 ± 2.43 a	-	10 plants	1.80 ± 0.36	-	10 females
Spiropidion	0.8	20.48 ± 5.23 ab	1	containing 3 cards with 60–90 mites each	0.75 ± 0.25	2	4 females	wbc	2	containing 3 cards with 60–90 mites each	0.78 ± 0.22	3	9 females
	1.2	27.91 ± 4.11 b	1		1.00 ± 0.00	2	4 females	34.11 ± 5.63 bc	2		1.25 ± 0.48	2	4 females
	1.6	26.46 ± 4.31 b	1		0.63 ± 0.32	3	8 females	28.57 ± 2.73 b	1		1.00 ± 0.31	2	7 females
Spirotetramat	1.34	30.71 ± 6.18 b	1		0.80 ± 0.49	2	5 females	38.11 ± 1.95 bc	2		1.29 ± 0.29	2	7 females
Imidacloprid	0.75	52.19 ± 5.85 c	2		-	-	-	39.66 ± 5.02 c	2		-	-	-
Statistics		$F_{3,52} = 7.24; P < 0.001$			$H = 3.45; P = 0.486$			$H = 22.72; P < 0.001$			$F_{4,32} = 1.73; P = 0.168$		

Lowercase letters within the same column stand for significant differences among treatments according to unifactorial ANOVA or Kruskal–Wallis ( $P < 0.05$ )

<sup>a</sup>International Organization for Biological Control toxicity categories for semi-field conditions, based on mortality or reduction of the parameter: 1 = harmless: < 25%; 2 = slightly harmful: 25–50%; 3 = moderately harmful: 51–75%; or 4 = harmful: > 75%

IOBC toxicological categories. Offspring of surviving adults did not differ among pesticides, and spiropidion and spirotetramat were harmless (Table 3). Results from persistent residue (8-day-old) followed the same trend than fresh residue, but mortality values were higher in general (Table 3). In consequence, spiropidion and spirotetramat were classified as innocuous, and imidacloprid as toxic. When comparing both residues, mortality was significantly higher at 8 days in all spiropidion doses.

## Discussion

The toxicity of the novel active ingredient spiropidion was evaluated on pupae and adults of *E. eremicus* and *N. tenuis* on topical and residual applications under laboratory and semifield conditions. In general, spiropidion is a slightly more selective compound than its counterpart spirotetramat for both natural enemies, a specific parasite and a generalist predator on *B. tabaci*. Spiropidion was more selective than spirotetramat, even at the highest doses, both in the emergence of treated pupae and in the survival of emerged adults during the first days for the whitefly parasitoid, *E. eremicus*. Mortality of adults emerging from pupae treated with 1.2 and 1.6 ml L<sup>-1</sup> of spiropidion and spirotetramat is slightly higher than in the control. This correlates with worse parasitizing ability. Mortality data remained on the edge of statistical significance, suggesting that an increase in the number of replicates would confirm the toxicity of both actives on the parasitoid. This pattern is repeated when adults of *E. eremicus* are exposed to 1 and 8 day-old residues in tomato plants, with the difference that the toxicity of the ketoenols is higher than when pupae are treated directly, but decreased in the case of imidacloprid, to which pupae was very sensitive. Spiropidion and spirotetramat are compatible with *N. tenuis*, whereas imidacloprid is not.

Pupae are the most resistant stage of *E. eremicus* and *Eretmocerus mundus* Mercet, after the evaluation of the toxicity of 24 different insecticides (IGR, neonicotinoids, synthetic pyrethroids, *Bacillus thuringiensis*, and so on) using the residual film method (for adults) and dipping method (for pupae) (Sugiyama et al., 2011). However, no tetrone/tetramic acid derivatives nor further parameters beyond mortality had been assayed until now. The use of immature

insects as well as adults demonstrated that the mortalities of both parasitoids depended on the type of insecticide and whether the parasitoid was in pupa or adult stage, which support the idea that different life stages must be evaluated (Fytrou et al., 2017). Gonzalez-Zamora et al. (1997, 2004) also found that *E. mundus* pupae seem to be less susceptible to insecticides since the pupal case of the whitefly constitutes a defensive barrier to the penetration of phytosanitary products. Francesena et al. (2017), when comparing spirotetramat with cypermethrin as positive control in a similar pupae treatment, hypothesized that both insecticides were able to penetrate the host cuticle and affect the parasitoid pupae, being spirotetramat less toxic due to its different mode of action. Thus, researchers agree in the results but not in the causes. Neonicotinoids seem to be the exception as most life traits of *Eretmocerus hayati* Zolnerowich and Rose F1 generation (development duration of immature, longevity of female adults, oviposition days and fecundity) were negatively affected by thiamethoxan (Guo et al., 2023), which is in accordance with the results reported herein for imidacloprid.

Despite spirotetramat is relatively safe to adult hymenopteran parasitoid of different families (Assouguem et al., 2021; Brück et al., 2009; Drobnjaković & Marčić, 2021; Garcerá et al., 2013; Tabebordbar et al., 2020), the profile of spiropidion is even better, being safe according to IOBC classification when adults are treated in laboratory. Spiropidion and spirotetramat exhibit low contact activity, but good systemic movement in the plant, since after foliar uptake, the insecticidal activity is translocated within the entire vascular system. Both are pro-insecticides with insecticidal activity against sucking pests because of the ambimobile behaviour: translaminar distribution and two-way xylem-phloem mobility of the spiropidion-enol, in the same way as spirotetramat-enol (Nauen et al., 2008); Brück et al., 2009; Muehlebach et al., 2020; Jeschke, 2021). In consequence, non-feeding plant insects as adults of *E. eremicus* should not be affected by contact exposure. However, in semifield bioassays, when adults walked on the surface of the treated plant, mortality increased slightly with respect to the topical trials and significantly compared with controls, and even more so with increasing age of the residue. This is an unexpected result, given the *E. eremicus* feeding behaviour, the mode of action of both compounds and way

**Table 3** *Nesidiocoris tenuis* adult mortality and offspring of surviving adults after run-off treatment of different insecticides (spiropidion, spirotetramat, imidacloprid) on tomato plants semi-field conditions

Treatment	Dose (ml L <sup>-1</sup> )	Fresh (1-day residue)			Persistence (8-day residue)								
		Mortality at 72 h		Nymphs per female		Mortality at 72 h		Nymphs per female					
		%	IOBC <sup>a</sup>	Replicates	Number	IOBC	Replicates	%	IOBC	Replicates			
Water-mock		4.25 ± 1.75 a	-	10 plants	71.30 ± 9.32	-	10 females	13.95 ± 2.56 a	-	10 plants	28.00 ± 5.52	-	11 females
Spiropidion	0.8	4.80 ± 2.58 a	1	containing 10 adults each	80.91 ± 8.26	1	12 females	21.18 ± 3.34 a	1	containing 10 adults each	24.20 ± 6.11	1	10 females
	1.2	3.00 ± 1.53 a	1	12 females	64.00 ± 7.41	1	12 females	18.55 ± 3.12 a	1	12 females	21.36 ± 6.45	1	11 females
Spirotetra- mat	1.6	5.30 ± 2.43 a	1	12 females	70.25 ± 11.08	1	12 females	19.26 ± 3.68 a	1	12 females	21.44 ± 5.13	1	9 females
	1.34	7.20 ± 2.18 a	1	9 females	59.11 ± 8.86	1	9 females	14.06 ± 3.72 a	1	9 females	33.77 ± 5.84	1	9 females
Imidacloprid	0.75	97.80 ± 1.51 b	3	-	-	-	-	100.00 ± 0.00 b	4	-	-	-	-
Statistics		$F_{5,54} = 345.43; P < 0.001$			$F_{4,50} = 0.80; P = 0.531$			$F_{5,48} = 146.97; P < 0.001$			$F_{4,45} = 0.75; P = 0.566$		

Lowercase letters within the same column stand for significant differences among treatments according to unifactorial ANOVA ( $P < 0.05$ )

<sup>a</sup>International Organization for Biological Control toxicity categories for semi-field conditions, based on mortality or reduction of the parameter: 1 = harmless: <25%; 2 = slightly harmful: 25–50%; 3 = moderately harmful: 51–75%; or 4 = harmful: > 75%

of treatment. It seems that both spirotetramat and spiropidion give rise to different metabolites in the degradation process that have been studied by different authors (Wu et al., 2023; Mahdavi et al., 2023). The latter quantified Spiropidion (SYN 546330) and their metabolites: spiropidion-enol (SYN 547305) as major metabolite, SYN 547435 and SYN 548430 with semi-quantitative analysis in multiple reaction monitoring mode (MRM) of UHPLC-MS/MS instrument for pistachio, resulting that after spraying, the main metabolite with the most concentration was spiropidion-enol. All metabolites increased to the 7th week, then reduced until the 10th week. It might be hypothesized that the metabolites were more toxic to the parasitoid than the initial compound, but there is no data on this.

Parasitization was significantly lower than that of the control for both, spiropidion and spirotetramat, when adults were treated. Similar effects have been reported on *E. mundus* for other compounds as diverse as abamectin, emamectin, deltamethrin, spirotetramat, metaflumizone (Fernández et al., 2010, 2015), azadirachtin, pyriproxifen and acetamiprid (Francesena & Schneider, 2018). Curiously, Yang et al. (2022) demonstrated that parasitic ability of *Encarsia formosa* Gahan (Hymenoptera: Aphelinidae) was inhibited upon heavy exposure to sublethal spirotetramat whereas, surprisingly, at light exposure induced hormesis and stimulated parasitic behaviour, which could assist in balancing chemical and biological control. On the other hand, when spiropidion or spirotetramat inhibit acetylCoA esterase, this interferes with lipid metabolism, reduces the amount of fatty acids needed for building new membranes during cell growth and development and, as a consequence, directly impacts on immature life stages and the fecundity of adult pest females (Muehlebach et al., 2020). The development of the resulting progeny was not followed in this work, but previous studies on *E. mundus* treated with spirotetramat showed that the insecticide impact all the life-history traits of *E. mundus* causing a significant disruption of the demographic parameters ( $r$ ,  $R_0$ , and  $T$ ). However, despite the sublethal effects reported, *E. mundus* was still able to contribute to the control of the host *B. tabaci* (Francesena et al., 2017).

Spirotetramat was completely harmless to *N. tenuis* after semifield treatment, in agreement with Wanumen et al. (2016) and Ziaei et al. (2017), who

evaluated label doses of six insecticides (pyriproxyfen, spirotetramat, cypermethrin, flubendiamide, metaflumizone and sulfoxaflor) and seven fungicides (benomyl, chlorothalonil, copper oxychloride, cyazofamid, fluopicolide + propamocarb hydrochloride (FPH), penconazol, trifloxystrobin). The tested insecticides and fungicides were all classified as harmless when predator was exposed only to the dry residues of each with the exception of metaflumizone classified as slightly harmless. Spiropidion, the new compound, showed identical toxicological profile than spirotetramat and most of the insecticides against the predator. However, Ziaei et al. (2017) also showed that when multiple exposure is tested, i.e., contact toxicity in combination with contaminated prey, some compounds initially innocuous may become toxic.

In the Mediterranean Basin, the occasional damage on tomato caused by *N. tenuis* appears to be outweighed by its role as BCA whereas in Northwest Europe, where *Macrolophus pygmaeus* Rambur (Hemiptera: Miridae) is the main BCA in greenhouse tomato crops, *N. tenuis* is considered an important emerging pest of tomato (Meesters et al., 2024). Thus, fourteen pesticides were assessed for their residual toxicity on adults and nymphs in laboratory experiments searching for insecticides that killed *N. tenuis* but were compatible with *M. pygmaeus*. Chlorantraniliprole, hexythiazox, tebufenozide and azadirachtin had minimal impact on *N. tenuis*, with less than 37.5% corrected mortality. Pirimicarb, sulfoxaflor, acetamiprid, flupyradifurone, thiacloprid, tebufenpyrad, spinosad, indoxacarb, emamectin benzoate and a mixture of pyrethrins and piperonyl butoxide were harmful to *N. tenuis*. This experiment, although it pursued the opposite of what is intended in this manuscript, showed that many of the insecticides used in greenhouses are not compatible with *N. tenuis* whereas both tetramic acids can be used jointly with the predator.

In our experiment, there was no option to negatively impact neither predator nor parasitoid feeding on intoxicated sap-sucking prey. Ziaei et al. (2017) did not observe lethal or sublethal effects on *N. tenuis* feed on spirotetramat treated eggs of *E. kuehniella*. Nevertheless, further experiments should be performed with the predator feeding on already intoxicated sap-sucking prey or directly on treated plant to confirm the lack of toxicity reported to date.

## Conclusions

Spiropidion apparently exhibits a favorable ecotoxicological profile, suitable for IPM practices, but more experiments must be carried out to have a complete picture of the potential impact of the compound and their metabolites on natural enemies, providing deeper insights into its pharmacokinetics and potential environmental impact. These developments are crucial as the European Union intensifies efforts to achieve a toxic-free environment under the European Green Deal.

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**Data availability** No datasets were generated or analysed during the current study.

## Declarations

**Compliance with ethical standards** This article does not contain any studies with human participants or animals performed by any of the authors.

**Informed consent for publication** All authors read and approved the manuscript.

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