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Compatibility of two novel insecticides dimpropyridaz and *Beauveria bassiana* PPRI 5339 with the beneficial insect *Nesidiocoris tenuis* (Reuter)

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ABSTRACT

Nesidiocoris tenuis is a zoophytophagous insect widely used commercially as a biological control agent against different pests and crops under greenhouse conditions. For a successful Integrated Pest Management (IPM) strategy, it is crucial to know the compatibility of available and new crop protection products with biological control agents. The aim of this study was to evaluate and classify the lethal and sublethal effects of different crop protection products on the biological control agent *N. tenuis*, including the novel insecticides dimpropyridaz and *Beauveria bassiana* strain PPRI 5339, in accordance with the International Organisation for Biological and Integrated Control (IOBC) directives. Dimpropyridaz, *B. bassiana* PPRI 5339, the adjuvant fatty acid esters and the combination of *B. bassiana* PPRI 5339 with the adjuvant have been classified as harmless (IOBC 1 $<$ 25% of mortality and beneficial capacity). The evaluation of reproduction and capacity for increase (r_c) showed no significant sublethal effects between the previous compounds and the untreated control. Flupyradifurone, sulfoxaflor, and dimethoate were classified as harmful (IOBC 4 $>$ 75% of mortality and beneficial capacity). The compatibility of these two new products (dimpropyridaz and *B. bassiana* PPRI 5339) with *Nesidiocoris tenuis* is of great importance because it adds new tools to be used in IPM programmes in which chemical and biological control strategies are used together.

1. Introduction

Integrated Pest Management (IPM) means the integration of all available plant protection methods in order to reduce populations of harmful organisms, keeping forms of intervention to levels that are economically and ecologically justified and reduce risks to human health and the environment. One of its principles is to protect and enhance the important beneficial organisms (European Commission, 2023). In this sense, IPM of current arthropod pests requires continuous investigation in the actual regulatory framework, where EU legislation (and from other economic regions and countries) is increasingly concerned about the impact of certain plant protection products on the environment (in the broadest sense) (European Commission, 2023). Crop protection products have been implicated on biodiversity impact, although their importance in controlling plant pest is clearly effective. Newly developed active ingredients or new formulations of existing ones go through evaluations regarding IPM compatibility (Passos et al., 2022). However, only those fulfilling the legal requirements are commercially implemented (Dáder et al., 2020; Handford et al., 2015; Maino et al., 2023; Villaverde et al., 2014). This means that although many active ingredients start at the beginning of the research process, very few of them are finally used in the field (Sparks, 2013). Innovation in crop protection is fundamental to having enough tools for farmers to use to protect crops against current and future pests in an environmentally sustainable manner (Sanz-Gomez et al., 2022).

Nesidiocoris tenuis (Reuter) (Hemiptera: Miridae) is a zoophytophagous insect that feeds on a broad pest spectrum, including whiteflies, thrips, leaf miners, aphids, spider mites and lepidopterans, with a high economic importance (Arnó et al., 2010; Mollá et al., 2014; Pérez-Hedo and Urbaneja, 2016; Sanchez, 2009; van Lenteren et al., 2021). *Nesidiocoris tenuis* is distributed worldwide and is commonly found in warm regions (Kerzhner and Josifov, 1999). Furthermore, it is widely commercially used as a biological control agent against whiteflies and other pests in greenhouse-grown tomatoes (Calvo et al., 2012).

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In the Mediterranean region, it is one of the most effective current biological control agents against whiteflies, such as *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae), but also against other species, including *Tuta absoluta* (Lepidoptera: Gelechiidae) (Arnó and Gabarra, 2011; Calvo et al., 2012; Dáder et al., 2020; Mollá et al., 2014; Pérez-Hedo and Urbaneja, 2016).

For the implementation of a successful IPM strategy, it is crucial to understand the compatibility of the available crop protection products against the target pest with potential biological control agents (Kaya and Keçeci, 2021). Within this context, the measurement of compatibility and side-effects of insecticides used to control different pests is necessary (Arnó and Gabarra, 2011). Earlier investigations showed the impact of insecticides on *N. tenuis,* regarding lethal and sublethal effects, by exposing *N. tenuis* to different compounds (Agrobío, 2022; Arnó and Gabarra, 2011; Dáder et al., 2020; Kaya and Keçeci, 2021; Kim et al., 2018; Koppert Biological Systems S.L, 2023; Wanumen et al., 2016). There is no information about the effects of dimpropyridaz and *Beauveria bassiana* PPRI 5339, two novel insecticides, on *N. tenuis*. Dimpropyridaz is a pyridazine pyrazolecarboxamides (PPCs). PPCs are a new class of chordotonal organ modulator insecticide for control of piercing–sucking pests, as many aphid species as well as whiteflies. This new family group inhibits chordotonal neurons and decreases intracellular Ca^{2+} levels, with the result that insects have uncoordinated movements and cannot feed and move properly. Moreover, the active metabolites originated from the active ingredient inside the insects have a different target site that other chordotonal hyper-active insecticides, which provides a novel mode of action (MoA 36) for resistance management (IRAC, 2023; Spalthoff et al., 2023). *Beauveria bassiana* is a widely present entomopathogenic fungus that can infest a variety of insects from different orders, with a high specifity. The infection pathway consists of, summarizing, in a penetration through the cuticle of the insect, overcoming the host response and immune defense reactions, and proliferation within the host by formation of yeast like cells, distributed passively in the hemolymph, with the invation of the host insect by extensive vegetative growth and the production of toxins; the fungus depletes nutrients in the hemolymph and the fat body, and this process is followed by the death of the insect with saprophytic outgrowth from the dead host and production of new conidia (Zimmermann, 2007). During the incubation period, the fungus may affect its host insect throughout behavioural and feeding changes, the reduction of body weight or fecundity, malformations or behavioural fever (Ekesi, 2001; Müller-Kögler, 1965; Ouedraogo et al., 2003, cited by Zimmermann, 2007)

Although *N. tenuis* is used as biological control, its phytophagy behaviour as a piercing–sucking insect is especially relevant regarding the new mode of action of dimpropyridaz (IRAC, 2023), as well as for *B. bassiana* PPRI 5339 due to its effective control on several pest species. The aim of this study was the evaluation and classification in laboratory trials of the lethal and sublethal effects of different crop protection products on the biological control agent *N. tenuis*, including the novel insecticides dimpropyridaz and *Beauveria bassiana* strain PPRI 5339, in accordance with the International Organisation for Biological and Integrated Control (IOBC) directives.

2. Materials and methods

2.1. *Nesidiocoris tenuis (Beneficial insect)*

The *N. tenuis* population tested was provided by Koppert Biological Systems S.L. under the commercial name Nesibug®. Second instar nymphs were received and used for the assays in the lab (Bakker et al., 2000)

2.2. *Insecticides*

Five different insecticides were used in the assays (Table 1). Dimpropyridaz is a new active ingredient discovered and developed by BASF and commercialised under the name AxaliOn™.

The active ingredient is a pyridazine pyrazolecarboxamide insecticide with a novel MoA 36 for the control of piercing and sucking insect pests and high compatibility with beneficial insects, including pollinators (IRAC, 2023; Moreno et al., 2022; Sanz-Gomez et al., 2022; Spalthoff et al., 2023). Furthermore, it has been shown to be an effective solution against insect vectors of some viruses, such as Beet Mild Yellowing Virus (BMYV) (Varrelmann et al., 2022). The same company has also launched Velifer®, an oil-based formulation of *Beauveria bassiana* strain PPRI 5339 (MoA UNF, IRAC, 2023) containing at least 8×10^9 viable conidia/ml (Cordero et al., 2021; Khun et al., 2020, 2021). *Beauveria bassiana* PPRI 5339 is naturally found (soil-borne fungus) and distributed worldwide; it is a parasite of various arthropod species, such as whiteflies (Aleyrodidae) or thrips (Thysanoptera), although it is being continually explored for management in other major risk areas (BASF, 2023).

As standard references, the active ingredients flupyradifurone (Sivanto®Prime) (MoA 4D) and sulfoxaflor (Closer®) (MoA 4C) were tested. These insecticides act on nicotinic acetylcholine receptor competitive modulators (IRAC, 2023). They are mainly used for the control of sucking insects. Both products are commonly used in greenhouses for the control of insect pests and particularly whiteflies (Dáder et al., 2020). Dimethoate (Perfekthion®) is a highly-toxic insecticide (Bostanian and Akalach, 2004; Kaya and Keçeci, 2021), and was used as a positive control. Dimethoate is an organophosphate insecticide. Its MoA classification is 1B, an inhibitor of acetylcholinesterase (AChE), causing hyperexcitation (IRAC, 2023). AChE is the enzyme that terminates the action of the excitatory neurotransmitter acetylcholine at

Table 1
Active ingredients tested for harmful effects on *Nesidiocoris tenuis* in laboratory trials.

Category	Trademark	Active ingredient	Manufacturer	Mode of Action Classification	Concentrations (g a.i. liter^{-1})	Formulation	Rate (%)[a]
Insecticide	Velifer®	*Beauveria bassiana* PPRI 5339	BASF SE, (Ludwigshafen, Germany)	UNF	80	OD	0.05
	AxaliON™	Dimpropyridaz	BASF SE, (Ludwigshafen, Germany)	36	120	SL	0.5
	Sivanto® Prime	Flupyradifurone	BAYER AG (Leverkusen, Germany)	4D	200	SL	0.075
	Closer®	Sulfoxaflor	CORTEVA Agriscience (Indianapolis, IN, USA)	4C	120	SC	0.2
	Perfekthion®	Dimethoate	BASF SE, (Ludwigshafen, Germany)	1B	400	EC	0.3
Adjuvant[b]	Break-Thru®SP 133	Fatty acid esters	EVONIK Industries AG (Essen, Germany)	–	100	SL	0.05

Abbreviations: ® (Registered Brand), ™ (Trademark), OD (Oil dispersion), SL (Soluble liquid), EC (Emulsifiable concentrate).

[a] Commercial rates recommended for whiteflies (water volume 200 l/ha), except dimethoate.

[b] Reduced rate.

nerve synapses (IRAC, 2023). All standard compounds were tested at the maximum field recommended concentration for tomato crop and whiteflies, according to product labels and the instructions provided by the Spanish Ministry of Agriculture, Fishing and Food (MAPA, 2023). Dimethoate was not registered for the tomato crop and whiteflies, so it was tested following the product label. *Beauveria bassiana* PPRI 5339 and dimpropyridaz were not registered when the experiments were carried out.

2.3. Adjuvant

One possible strategy to improve *B. bassiana PPRI 5339* performance is the addition of emulsifiable oils or other adjuvants in conidial suspensions (Akbar et al., 2005; Gatarayiha et al., 2010). Therefore, Break-Thru®SP 133 (fatty acid esters) solo and in addition to *B. bassiana* PPRI 5339 formulation were included in this study (Table 1).

2.4. Lethal and sublethal effects on N. tenuis

Active ingredients are generally evaluated in a step by step process through levels of sequential decision-making to obtain a classification. In the present work, the experiments started at level I, which evaluates the residual toxicity on juveniles to a fresh residue of the product in the laboratory. Depending on the effect results, it is proceeded regarding some scenarios. With low toxicity results (below the lower threshold), no further testing would be needed for classification. With an effect between the upper and lower thresholds it would proceed to the next level, persistence effect. The effect above the upper threshold means that the product has such high toxicity that no further testing is needed for classification (EPPO, 1998).

The experiments to assess the lethal and sublethal effects of the products consisted of two phases. The first phase determined the lethal effects of different compound residues on young *N. tenuis* nymphs. The second phase was developed to study the sublethal effects of the active ingredients on the offspring of the adults moulted in the previous phase and was extended to include the effects on the survival of female adults and their capacity for increase. To confirm the obtained results, the experiments were replicated twice. The assays were carried out under laboratory conditions, adapting the methodologies described by Bakker et al. (2000), Hassan et al. (1985), and EPPO PP 1/151 (2) (EPPO, 1998). Young nymphs of the second instar, used as the most sensitive stage of the insect, were exposed to fresh residues of the tested compounds on eggplant leaf discs of 7 cm diameter placed on top of a wet cotton piece to prevent leaf dehydration. The leaf discs were treated in a spray chamber with a fan nozzle (XR Teejet 110015 VS). All insecticides were used at the commercial rates recommended for whitefly control (Table 1), simulating a water spray volume of 200 l/ha, applied at 3 bars of pressure. The control leaf discs were treated with osmotised water. Once the leaf discs were dry (1 h after treatment), ten *N. tenuis* second instar nymphs (one replicate) were placed into the experimental unit.

A methacrylate cylinder (7 cm diameter and 3.7 cm height) covered with Fluon® (fluoropolymer resin (PTFE)) on the inner face was used to prevent *N. tenuis* from climbing out of the cylinder and therefore ensure contact with residues throughout the duration of the experiment. The cylinders were closed with a lid that had a 4 cm hole covered with a mesh to allow ventilation within the experimental unit. As a food source for *N. tenuis*, 0.05 g of *Ephestia kuehniella* eggs and *Artemia* spp cysts commercialised as Entofood® were placed on the centre of each leaf disc after application. An additional 0.025 g of food per leaf disc was provided four days later. The experimental units were maintained throughout the duration of the study in a climate-controlled room at 25 °C with 60% relative humidity (RH) and a photoperiod of 16:8 h (L:D).

The alive and dead nymphs were recorded 1, 4 and 7 days after they were placed on treated leaf discs. Insects were considered dead when they were touched with a brush and no movement was observed. Five replications per active ingredient were carried out, which means a total of 50 nymphs per active ingredient and experiment.

Seven days after treatment, the second phase was carried out to evaluate the sublethal effects on reproduction. This second phase of the study was performed using treatments with low mortality in the previous phase, namely dimpropyridaz, *B. bassiana* PPRI 5339, *B. bassiana* PPRI 5339 + adjuvant and adjuvant solo. The adults that moulted from the surviving nymphs were removed and placed on new untreated eggplant leaf discs to study the sublethal effects on offspring at four consecutive 3–4-day intervals. The first and third intervals had a period of 4 days, and the second and fourth intervals had 3 days. In total, the sublethal effect was studied over a 14-day period. The experimental unit used was the same as for the first phase. Three females and 2 males were placed on the leaf disc to allow oviposition. When a female died, this was replaced by a new one from a pool of individuals treated in phase one to maintain 15 alive females per treatment (Bakker et al., 2000). At the beginning of each interval, leaf discs were replaced by a fresh disc to allow further oviposition, and the previous discs were maintained to evaluate the number of emerged nymphs. The total number of nymphs that emerged per female and day over the four intervals and the 14-day period was used to analyse the effect of the compounds on the offspring. Five replications per treatment were carried out.

Additionally, the capacity of increase was calculated. Biologically, this parameter is the number of times the population will multiply per unit of time (Birch, 1948). Two parameters were evaluated: a) the average daily offspring (as females) per female (m_x) in the four intervals and b) the number of adult females that died on each evaluation day until the experiment was finished, which, in conjunction with nymph survival from the first phase, was used to calculate l_x throughout the experiment. Both parameters (m_x and l_x) were used to calculate r_c (capacity of increase, an approximation of the intrinsic rate of natural increase r_m), following the procedure in Southwood and Henderson (2000) for each of the active ingredients tested, being the net reproductive rate R_0 ($R_0 = \sum_x l_x m_x$) and capacity of increase r_c, ($r_c = \ln R_0 / T_c$), where T_c is the cohort generation time (mean age of the females in the cohort when half of the female offspring are produced ($T_c = 0.5\ R_0$)). The values of m_x were calculated using the female proportion in the offspring (0.524), obtained as an average from different references (Baños-Díaz et al., 2017; Mollá et al., 2014; Sanchez, 2009) and the preadult period (23.2 days) was also obtained as an average from the references available (Baños-Díaz et al., 2017; Ebrahimi et al., 2019; Sanchez et al., 2009; Yano et al., 2014, 2020). There were 10 values of r_c at the end of the calculations (two values for each of the four products tested plus the control, obtained from the two experiments made), and the Jackknife re-sampling procedure was used to generate pseudovalues for each r_c value and to calculate the mean and its associated standard error.

Females that died on each evaluation day in the two experiments (second phase) were used to compare the adult survival in each compound tested, applying a proportional hazards analysis (Fox and Weisberg, 2011) also called the Cox model, which has been used successfully in medical research to study mortality and in the entomological field, for example, in Haccou and Hemerik (1985), as cited in van Alphen et al. (2003) or in Bareil et al. (2018).

Two identical experiments (experiments 1 and 2) were conducted to confirm the results and to obtain enough data for analysis. Experiment 1 started 14th November and finished 16th December 2019. Experiment 2 was conducted from 9th January to 10th February 2020.

2.5. Data analysis

The data from phase one of both experiments were statistically evaluated using a two-way analysis of variance to analyse the homogeneity of the effect of the products on *N. tenuis* (Treatment factor), as well as the homogeneity of the experiments (Experiment factor). Tukey's test was used in the analysis for mean separation at $p < 0.05$. If the interaction of both factors differed significantly from homogeneity, a one-way analysis of variance was evaluated separately for both experiments. Mortality values were transformed into the arcsin square root.

Because the offspring were evaluated in four intervals, a repeated measures analysis of variance was applied for the effect on the compound exposure using time. The assumption of sphericity was tested with Mauchly's test, which applied the correction of the degrees of freedom when necessary. If a significant effect was found in the Treatment factor, groups were compared with Tukey's test. Nymph mortality caused by active ingredients was corrected according to Abbott's formula (Abbott, 1925): $M = 100 \times ((Mt - Mc)/(100-Mc))$, where M is the corrected mortality, Mc is the control mortality and Mt is the treatment mortality. The reduction of the beneficial capacity (E) was calculated by means of the following equation (Overmeer and van Zon, 1982): $E = 100 - ((100-M)x(Rt/Rc))$, where M is the corrected mortality, Rc is the mean reproductive performance of the control and Rt is the mean reproductive performance of the treatments. Subsequently, data were interpreted according to the IOBC toxicity categories for extended laboratory tests of residual effects (Sterk et al., 1999): (1) harmless (<25% mortality), (2) slightly harmful (25–50% mortality), (3) moderately harmful (51–75% mortality) and (4) harmful (>75% mortality).

The capacity for increase (r_c) was analysed using a two-way (factors Treatment and Experiment) analysis of variance, and Tukey's test was used to separate groups if a significant effect was found. Adult female survival was analysed for Treatment and Experiment effects with Cox proportional hazards analysis.

Statgraphics Centurion 18.1.16 and SPSS 15.0 (for repeated measures ANOVA and Cox procedure) were used for the data analyses.

3. Results

The survivorship and mortality caused by the different active ingredients were tested on second instar nymphs of *N. tenuis* in the first phase 1, 4 and 7 days after residual exposure. Statistical differences were found in the interaction between trials and products, both in alive nymphs (with $P < 0.001$; $P = 0.001$; $P = 0.016$, 1, 4 and 7 days after treatment, respectively) and percentage of mortality (with $P < 0.001$; $P = 0.001$; $P = 0.016$, 1, 4 and 7 days after treatment, respectively). Therefore, both experiments were considered and analysed separately (Table 2). In all cases, dimpropyridaz and *B. bassiana* PPRI 5339 showed low mortality, with no statistical differences with respect to the untreated control in all evaluations of both experiments. The same results were obtained for the adjuvant solo and its mixture with *B. bassiana* PPRI 5339. It was not observed the typical effects of PPCs (as dimpropyridaz) on target pest species, like uncoordinated movements, and in the *B. bassiana* PPRI 5339 treatment no signs of infection was observed either (as fungus growth and production of conidia). In contrast, flupyradifurone, sulfoxaflor and dimethoate showed significant negative effects on the survivorship of the nymphs at 4 and 7 days after exposure to the compounds. Dimpropyridaz, *B. bassiana* PPRI 5339, *B. bassiana* PPRI 5339 + adjuvant and adjuvant solo were classified as harmless according to the IOBC, while flupyradifurone, sulfoxaflor and dimethoate were classified as harmful (Table 3).

When the effect of mortality on nymphs and total offspring per female production were combined (beneficial capacity) (Table 3), the group of active ingredients that overcame the first phase experiments did not show a reduction in the beneficial capacity of *N. tenuis* compared with the control and therefore were ranked as harmless according to IOBC categories.

The reproduction evaluation in phase two of the study (Table 4) showed no significant sublethal effects among the tested compounds and the untreated control. A lower number of offspring were observed in

Table 3
IOBC Toxicity categories obtained for the different active ingredients studied on *Nesidicoris tenuis* in laboratory trials.

Active ingredients	% Mortality (corrected with the Abbott's formula) (Mean ± SE)[†] 7 DAA	IOBC Toxicity categories	% Reduction of the beneficial capacity (Mean ± SE)[†]	IOBC Toxicity categories
Control	0.0 ± 0.0 a		0.0 ± 0.0 a	
B. bassiana PPRI 5339	−3.8 ± 5.0 a	1	−28.8 ± 12.4 a	1
B. bassiana PPRI 5339 + Fatty acid esters	−0.3 ± 4.3 a	1	−30.3 ± 15.1 a	1
Fatty acid esters	−1.9 ± 4.3 a	1	−18.8 ± 16.8 a	1
Dimpropyridaz	−6.2 ± 5.1 a	1	−24.8 ± 21.1 a	1
Flupyradifurone	89.7 ± 4.5 b	4	100.0 ± 0.0 b	4
Sulfoxaflor	100.0 ± 0.0 b	4	100.0 ± 0.0 b	4
Dimethoate	100.0 ± 0.0 b	4	100.0 ± 0.0 b	4
F; df; P (A)	221.55; 7, 63; <0.001		25.20; 7, 63; <0.001	
F; df; P (B)	10.58; 1, 63; 0.002		0.09; 1, 63; 0.768	
F; df; P (A) x (B)	1.51; 7, 63; 0.179		0.23; 7, 63; 0.977	

Abbreviations: SE (Standard error), F (F-value), df (degree of freedom), P (P-value); A (Active ingredient); B (Experiment); IOBC (International Organisation for Biological and Integrated Control).

†Within columns, means followed by the same letter do not differ significantly (Tukey's test; P > 0.05). Negative values indicate that the treatments performed better than the control.

Table 2
Percentage of mortality of *Nesidiocoris tenuis* nymphs 1, 4 and 7 days after residual exposure in the two experiments carried out.

Active ingredients	Mortality (%) (mean ± SE)[a]					
	1 DAA		4 DAA		7 DAA	
	EXP 1	EXP 2	EXP 1	EXP 2	EXP 1	EXP 2
Control	4.0 ± 2.4 cd	0.0 ± 0.0 c	12.0 ± 5.8 c	0.0 ± 0.0 b	18.0 ± 5.8 b	4.0 ± 2.4 b
B. bassiana PPRI 5339	2.0 ± 2.0 d	0.0 ± 0.0 c	2.0 ± 2.0 c	4.0 ± 2.4 b	8.0 ± 3.7 b	10.0 ± 5.5 b
B. bassiana PPRI 5339 + Fatty acid esters	4.0 ± 2.4 cd	0.0 ± 0.0 c	10.0 ± 6.3 c	4.0 ± 2.4 b	16.0 ± 8.1 b	6.0 ± 4.0 b
Fatty acid esters	2.0 ± 2.0 d	2.0 ± 2.0 c	4.0 ± 2.4 c	6.0 ± 2.4 b	14.0 ± 6.8 b	6.0 ± 2.4 b
Dimpropyridaz	2.0 ± 2.0 d	2.0 ± 2.0 c	10.0 ± 3.2 c	4.0 ± 2.4 b	10.0 ± 3.2 b	4.0 ± 2.4 b
Flupyradifurone	14.0 ± 2.4 bc	58.0 ± 7.3 b	84.0 ± 4.0 b	100.0 ± 0.0 a	84.0 ± 4.0 a	100.0 ± 0.0 a
Sulfoxaflor	32.5 ± 11.1 b	68.0 ± 7.3 b	100.0 ± 0.0 a	100.0 ± 0.0 a	100.0 ± 0.0 a	100.0 ± 0.0 a
Dimethoate	100.0 ± 0.0 a	100.0 ± 0.0 a	100.0 ± 0.0 a	100.0 ± 0.0 a	100.0 ± 0.0 a	100.0 ± 0.0 a
F; df; P	58.90; 7, 38; <0.001	139.61; 7, 39; <0.001	64.12; 7, 38; <0.001	183.49; 7, 39; <0.001	42.82; 7, 38; <0.001	103.82; 7, 39; <0.001

Abbreviations: SE (Standard error); DAA (Days after application); EXP (Experiment), F (F-value), df (degree of freedom), P (P-value).

[a] Within columns, means followed by the same letter do not differ significantly (Tukey's test; P > 0.05).

Table 4

Nesidiocoris tenuis offspring (nymphs/female/day) in the four intervals considered.

Active ingredients	Nymphs/female/day (Mean ± SE)[†]					
	Interval 1	Interval 2	Interval 3	Interval 4[‡]		Intervals 1,2,3
				EXP 1	EXP 2	
Control	5.3 ± 0.5 a	6.6 ± 1.2 a	6.0 ± 0.6 a	6.3 ± 1.0 a	4.0 ± 1.8 a	6.0 ± 0.7 a
B. bassiana PPRI 5339	6.5 ± 0.6 a	7.2 ± 1.2 a	7.4 ± 0.8 a	7.2 ± 0.8 a	6.8 ± 0.5 a	7.0 ± 0.6 a
B. bassiana PPRI 5339 + Fatty acid esters	5.4 ± 0.6 a	7.8 ± 1.1 a	8.7 ± 0.9 a	8.9 ± 0.8 a		7.3 ± 0.6 a
Fatty acid esters	4.5 ± 0.4 a	7.2 ± 1.1 a	7.5 ± 0.7 a	9.7 ± 1.1 a	5.1 ± 0.4 a	6.5 ± 0.6 a
Dimpropyridaz	5.0 ± 0.8 a	6.7 ± 1.0 a	6.4 ± 0.7 a	7.2 ± 0.9 a	7.4 ± 2.3 a	6.0 ± 0.6 a
F; df; P (A)	1.57; 4, 40; 0.201	0.46; 4, 40; 0.768	2.34; 4, 38; 0.073	2.09; 4, 18; 0.137	1.07; 3, 8; 0.415	1.56; 4, 38; 0.204
F; df; P (B)	0.01; 1, 40; 0.909	67.64; 1, 40; <0.001	5.70; 1, 38; 0.022			31.00; 1, 38; <0.001
F; df; P (A) x (B)	0.48; 4, 40; 0.7495	0.23; 4, 40; 0.9218	1.52; 4, 38; 0.2167			1.07; 4, 38; 0.3858
Repeated measures: F; df; P (A)						1.024; 4, 45; 0.405
F; df; P (C)						9.79; 2, 90; 0.000

Abbreviations: SE (Standard error), EXP (Experiment), F (F-value), df (degree of freedom), P (P-value); A (Active ingredient); B (Experiment); C (Interval).

[†] Within columns, means followed by the same letter do not differ significantly (Tukey's test; P > 0.05).

[‡] Interval 4a was separated in the two experiments due to the total mortality in the *B. bassiana* PPRI 5339 + adjuvant replica in experiment 2.

interval 1 in comparison with intervals 2 and 3 (significant differences between periods $P < 0.001$), but this reduction was attributed to the beginning of oviposition. Flupyradifurone, sulfoxaflor and dimethoate were not included in this phase due to the high mortality shown in phase one of the experiments.

Adult female survival during the second phase of the study showed differences between the two experiments ($P = 0.005$, with more survival in the second experiment). Furthermore, the interaction between the experiment and product was significant ($P = 0.001$) for the adjuvant (less survival than the control in the second experiment) and its mixture with *B. bassiana* PPRI 5339 (more survival than the control in the second experiment). When the analysis was performed considering the two experiments together, the products tested showed no significant effect on female survival compared to the untreated control ($P = 0.823$, Fig. 1).

The capacity of the population increase of *N. tenuis* (r_c, Table 5) represents an integral value of the potential of increasing its population from birth until the death of the adults (or the end of the experiment). Both experiments resulted in similar values of r_c in the principal products (*B. bassiana PPRI* 5339, *B. bassiana PPRI* 5339 + adjuvant, adjuvant solo and dimpropyridaz) and only in experiment 1 was the r_c of the adjuvant (0.102 ± 0.005) significantly lower than that of the other products (*B. bassiana* PPRI 5339, 0.121 ± 0.003). Considering together the r_c pseudovalues of the two experiments (to gain a general view), the four ingredients tested showed similar values to the control (Table 5).

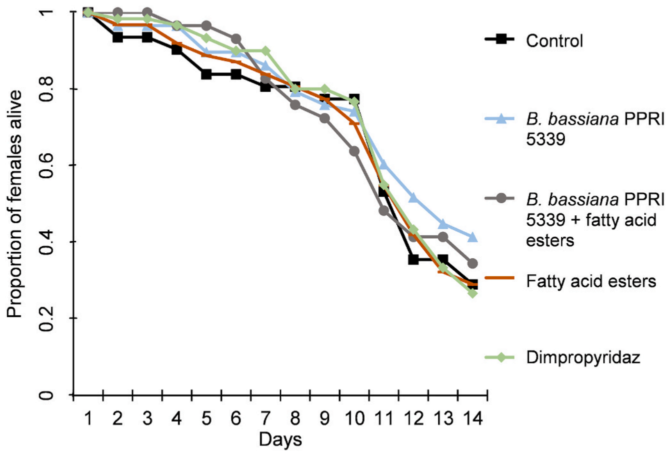

Fig. 1. Evolution of female adult survival considering together the two experiments in the second phase of the study (offspring production).

Table 5

Capacity of population increase (r_c) for *Nesidiocoris tenuis* in the two experiments and the pseudovalues obtained for experiments separately and combined.

Active ingredients	Capacity for increase (r_c) (mean ± SE)[†]				
	Raw data (mean)		Pseudovalues (mean ± SE)		
	EXP 1	EXP 2	EXP 1	EXP 2	EXP 1 & EXP 2
Control	0.106	0.123	0.107 ± 0.005 ab	0.126 ± 0.005 a	0.117 ± 0.005
B. bassiana PPRI 5339	0.121	0.124	0.121 ± 0.003 a	0.123 ± 0.003 a	0.122 ± 0.002
B. bassiana PPRI 5339 + Fatty acid esters	0.115	0.124	0.116 ± 0.005 ab	0.120 ± 0.001 a	0.118 ± 0.003
Fatty acid esters	0.103	0.127	0.102 ± 0.005 b	0.127 ± 0.002 a	0.114 ± 0.005
Dimpropyridaz	0.114	0.121	0.114 ± 0.003 ab	0.121 ± 0.007 a	0.118 ± 0.004
F; df; P (A)			3.20; 4, 20; 0.035	0.57; 4, 20; 0.687	0.98; 4, 40; 0.431
F; df; P (B)					17.89; 1, 40; <0.001
F; df; P (A) x (B)					2.92; 4, 40; 0.033

Abbreviations: SE (Standard error), EXP (Experiment), F (F-value), df (degree of freedom), P (P-value); A (Active ingredient); B (Experiment).

[†] Within columns, means followed by the same letter do not differ significantly (Tukey's test; P > 0.05).

4. Discussion

Biological control is an important IPM tool, as well as chemical control, and working together can ensure better crop protection, reducing applications (in consequence residues), and improving resistance management. The use of beneficial insects demands active ingredients that are harmless for them and efficient for pest control. In this context, the new active ingredients and formulations tested in the experiments presented here showed a clear compatibility with the mirid *N. tenuis* in all tests carried out: nymph survival in contact with residues, offspring production of the females (and the combination of the two variables in the beneficial capacity), adult female survival, and the overall performance considered in the parameter r_c. In all parameters, the products tested were significantly similar to the control (if not better), indicating that no negative effects were produced by the active ingredients (dimpropyridaz, *B. bassiana* PPRI 5339, *B. bassiana* PPRI5339 + adjuvant and adjuvant solo) during the study.

4.1. Lethal effect

In the first phase, 82% of nymph survival was reached in the control plots of experiment 1, similar to Ebrahimi et al. (2019) with a preadult survival rate of 0.8, and Mollá et al. (2014) with 89% survival. In the control plots of experiment 2, 96% of the nymphs survived, similar to Yano et al. (2020) (100%) and Sanchez et al. (2009) (0.065 nymphal mortality).

Dimpropyridaz and *B. bassiana* PPRI 5339 were classified as harmless (IOBC 1) for *N. tenuis* in both the corrected mortality of nymphal instars and in the effect on the beneficial capacity. There is not much information published about the new products studied here (particularly dimpropyridaz) in relation to our study. *Nesidiocoris tenuis* has in common with target pest of dimpropyridaz the order and therefore the chordotonal organ modulator, however the results in our study showed no lethal effect on *N. tenuis*, explained because not all pests, or insect stages, are equally susceptible (Prabhaker et al., 2006; Sayed et al., 2021). IOBC classes are useful for comparing research data but it is also quite important to further investigate sublethal effects in order to assess the overall side effects of a chemical on non-target organisms (Roditakis et al., 2014), as presented in our study. Regarding information about other strains of *B. bassiana* and its effect on diverse beneficial insects, similar results to the present study were found, for example Agrobío (2022) classified *B. bassiana* GHA (Botanigard ®) as harmless (IOBC 1) on *N. tenuis*, Hamdi et al. (2011) concluded that second instar larvae of *Macrolophus caliginosus* were not susceptible to *B. bassiana* ATCC (Naturalis®-L) after any mode of contamination, by spraying directly on larvae (85% of survival), on the foliar feeding substrate (97.1% survival) or by contaminated *Trialeurodes vaporariorum* prey (88.1–91.7% of survival, depending on fungus inoculation timing). Jacobson et al. (2001) suggested that *B. bassiana* ATCC (Naturalis®-L) could be used as a second line of defence to support preventive pest management with *Amblyseius cucumeris* (0% of mortality of the predator). Sayed et al. (2021) concluded that an indigenous *B. bassiana* (indigenous isolate) did not affect *Coccinella undecimpunctata* (mortality lower than 20% in different stages) or *Hippodamia variegate* (mortality lower than 21% in different stages). However, Shipp et al. (2003) did not recommend introducing *Orius insidiousus* during the application of *B. bassiana* GHA (Botanigard® ES) (60.9 and 76.9% of infection on adults, 15.5 and 42.2% of infection on immatures, at 75 and 97.5% RH, respectively). Zimmermann (2007) did a review of examples of the effects of *B. bassiana* on beneficial organisms, including Donegan and Lighthart (1989), demonstrating that some factors affected the susceptibility of *Chrysoperla carnea* to *B. bassiana,* such as temperature, starvation, and nutrition stress. It is well known that *B. bassiana* is a generalistic entomopathonic fungus that infects phytophagous insects and some beneficial insects, but the same fungus isolate could have diverse effects on different hosts, even if they are from the same family (Sayed et al., 2021), so it could explain the compatibility of *B. bassiana* PPRI 5339 with *N. tenuis*.

The adjuvant (Break-Thru® SP 133) was studied as a strategy to improve the effectiveness of *B. bassiana* PPRI 5339 (Velifer®) for the control of target pests. Adjuvants are not subjected to regulatory risk assessment; consequently, little public information relative to this topic is available (Wernecke et al., 2022). In our studies, it was classified as harmless (IOBC 1) on *N. tenuis*, solo or in combination with *B. bassiana* PPRI 5339. Regarding the remaining parameters, this adjuvant had the same profile as the control.

Flupyradifurone and sulfoxaflor were included in our studies as active ingredients commonly used in greenhouses for pest control (particularly whiteflies). Based on our results, flupyradifurone and sulfoxaflor were classified as harmful (IOBC 4). These insecticides act on nicotinic acetylcholine receptor competitive modulators and they are mainly used for the control of sucking insects and it could explain our results. They were similar to data available both on the internet and published. On the web page of Koppert Biological Systems S.L, (2023) sulfoxaflor appears classified as harmful (IOBC 4) and flupyradifurone as moderately harmful (IOBC 3). Wanumen et al. (2016) concluded that sulfoxaflor was harmful to *N. tenuis* and *Macrolophus basicornis*, and Kim et al. (2018) obtained 100% mortality on *N. tenuis* 48 h after direct application. Other authors have found that sulfoxaflor showed more than 70% mortality in a hemipteran species (*Orius* sp.*)* (Barbosa et al., 2017; Dáder et al., 2020; Kim et al., 2018). Barbosa et al. (2017) included flupyradifurone in their study and showed 60.5% mortality for *Orius insidiosus*. These two active ingredients should follow the next level of sequential decision-making for classification (EPPO, 1998), level II, persistence test. Some studies have demonstrated high residue ageing, for example Wanumen et al. (2016) showed long residual activity and high toxicity until 34 days after treatment for sulfoxaflor.

Dimethoate was studied as a toxic reference. In our study, dimethoate was classified as harmful (IOBC 4), with similar results to Kaya and Keçeci (2021). Other references have classified dimethoate as harmful according to the IOBC criteria in other beneficial organisms, such as *Orius* sp. or *Macrolophus caliginosus* (Alzoubi and Çobanoğlu, 2010; Angeli et al., 2005; Bostanian and Akalach, 2004; Tedeschi et al., 2002).

4.2. Sublethal effect

Regarding the sublethal effect, in the second phase of our studies, dimpropyridaz and *B. bassiana* PPRI 5339 had the same performance as the control; they did not reduce the number of offspring (nymphs per female) or the capacity for increase (r_c). The comparison of our offspring results with other authors depends on pre-oviposition, oviposition period, or adult female longevity values. In our study, for intervals 1, 2 and 3, we found 66 ± 7.7 nymphs per female on 11 days, similar to Ebrahimi et al. (2019) (117.3 ± 11.66 in 19 days) but higher than Sanchez et al. (2009) (60 ± 5.0 in 21 days) and Perdikis and Lykouressis (2002) with *Macrolophus pygmaeus* (162.2 ± 8.3 in 63.7 days). This difference could be due to the oviposition substrate, feeding prey or the methodology followed in each study (Nakaishi et al., 2011).

The survival analysis of female adults (females moulted from the nymphs tested in the first phase) by the Cox regression model showed that the main active ingredients (*B. bassiana* and dimpropyridaz) were similar to the control.

The capacity of increase (r_c) is a parameter that integers all variables that define a population's potential (nymphal mortality, sex ratio, daily offspring per female, time to peak offspring production, and adult female survival) and gives a complete view of the potential population increment under certain environmental restrictions, similar to the application of different active ingredients used in this study. The value obtained in the control for the conjunct of our study was similar to many of the values found in the literature, such as in Gavkare et al. (2021) ($r_c = 0.1050$), Baños-Díaz et al. (2017) ($r_m = 0.111$), Mollá et al. (2014) ($r_c = 0.112 \pm 0.001$), and Yano et al. (2014) ($r_m = 0.1096$), all of which used *Ephestia kuehniella* eggs as food, as in our study. This highlights the good performance of the experiment and the lack of detrimental effects found on the *N. tenuis* population throughout the study, both in the control and in some of the products tested.

In other cases, the value of r_c (or r_m) was more different (with a lower or higher value) than in our study, depending mainly on the type of food used, the rearing substrate, method of calculation, or for other reasons, such as in Nakaishi et al. (2011) ($r_m = 0.0855$, with *E. kuehniella* eggs but sesame leaves as substrate), Yano et al. (2020) ($r_m = 0.128 \pm 0.005$ with *Bemisia tabaci* as food, and $r_m = 0.063$ with *Thrips palmi* as food), and Ebrahimi et al. (2019) ($r_m = 0.136 \pm 0.005$, with eggs of *Plutella xylostella*).

As a final consideration, the present study was conducted with laboratory trials, but following research should provide information of its effect on *N. tenuis* when it is feeding and developing on treated *B. tabaci* under laboratory conditions or in more realistic (as semifield and field) trials in order to have a complete view of the products.

5. Conclusions

Our study provides new important information about the side effect and compatibility of two new insecticides recently developed, *B. bassiana* PPRI 5339 (Velifer®) (alone and together with the fatty acid esters adjuvant Break-Thru®SP 133) and dimpropyridaz (AxaliOn™), on *N. tenuis* nymphs. Both active ingredients (and the adjuvant) have shown their compatibility with the juvenile instars of *N. tenuis* and the population development of this important predator in the laboratory. The compatibility of these two new products with *N. tenuis* is of great importance because it adds new tools to be used in IPM programmes, in which chemical and biological control strategies are used together.

CRediT authorship contribution statement

Estefanía Rivera-Alonso: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Resources, Visualization, Writing – original draft, Writing – review & editing. **José E. González-Zamora:** Formal analysis, Visualization, Writing – original draft, Writing – review & editing. **Carlos Avilla:** Conceptualization, Writing – review & editing. **Jorge Sanz-Gomez:** Conceptualization, Resources, Supervision, Writing – review & editing.

Declaration of competing interest

Estefanía Rivera-Alonso is actually working in the Agricultural Research Station of BASF Española. S.L. at Utrera (Spain), as Insecticides Indication Manager. All the research presented in the manuscript was carried out in the research station.

Data availability

The data that has been used is confidential.

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