Research Article

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Response of *Amblyseius swirskii* to deltamethrin

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Abstract

BACKGROUND: The rising demand for environmentally friendly pest control highlights the importance of understanding the interaction between natural enemies and pesticides. *Amblyseius swirskii*, a predatory mite extensively used in biocontrol, plays a crucial role in managing pest populations in agricultural systems. Integrating this mite with selective pesticide use within integrated pest management (IPM) would significantly advance pest control and may reduce pesticide residues in the environment and agricultural produce.
This study characterized the susceptibility of two *Amblyseius swirskii* colonies to deltamethrin, a widely used pesticide, to assess their potential integration into IPM strategies.

RESULTS: Both colonies exhibited significant tolerance to deltamethrin at concentrations higher than the maximum recommended field rate. Our analysis identified mutations in the target site in both populations. The commercial population also showed a contribution of cytochromes P450 to the resistant phenotype. Despite these results, semi-field trials revealed a significant reduction in mite counts post-treatment with deltamethrin; various experiments were conducted to understand this discrepancy.

CONCLUSION: This study underscores the need for comprehensive evaluations of pesticide impacts on biological control agents to optimize IPM strategies. Understanding pesticide resistance and field performance dynamics is crucial for developing sustainable pest management practices that ensure environmental resilience and agricultural productivity.

Supporting information may be found in the online version of this article.

Keywords: integrated pest management (IPM); pesticide resistance; biological control; phytoseiids; pyrethroids

1 INTRODUCTION

Biological control agents (BCAs) are key components of integrated pest management (IPM) programs. Unlike methods that rely heavily on chemical use, BCAs provide a sustainable and safe approach to managing pest populations. IPM is strongly supported by the European Union, with the clear objective of achieving sustainable use of pesticides to reduce it in favor of biological control alternatives.[1–3] A prime example of successful biological control program implementation is shown in the Almería region (Spain), where the area dedicated to these programs expanded dramatically from 515 ha in the 2006–2007 season to 26 288 ha in the 2020–2021 season.[4]

Predatory mites, especially those from the Phytoseiidae family, are essential for the success of augmentative biological control.[5,6] They are predominantly used in protected vegetable and ornamental crops to manage phytophagous mites, thrips, and whiteflies. However, they still have limited use in open-field systems.[7] One of the most important predatory mites is the polyphagous species *Amblyseius swirskii* Athias Henriot (Mesostigmata: Phytoseiidae).[8] This mite is used to control whiteflies and thrips, but it can also feed on tetranychid species, broad mites, and, in some cases, on honeydew.[7,9–11]

Pyrethroids are extensively used in agriculture, veterinary, and household applications, constituting a significant portion of the pesticide market.[12] While their broad-spectrum efficacy and favorable ecotoxicological profile make them highly valued, their

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interaction with beneficial arthropods, particularly phytoseiid predatory mites, is notably high. This sensitivity can impact the balance of biological control systems where these mites play a crucial role, underscoring the need to carefully assess and manage pyrethroid applications in IPM contexts. These compounds target the voltage-gated sodium channel (VGSC), a fundamental protein initiating and propagating action potentials in their nervous system.[13] However, prolonged and intensive usage has spurred the evolution of resistance in pest species. In arthropods, common resistance mechanisms to pyrethroids include metabolic resistance,[14,15] characterized by the up-regulation of detoxification enzymes and target-site insensitivity caused by substituting key residues in the VGSC.[16,17] These substituted residues are predominantly found within the linker between transmembrane segments four and five of domain II (IIS4-S5), as well as in segments five (IIS5) and six (IIS6) of the same domain, and segment six of domain III (IIIS6).[18] Although there are cases of resistance linked only to one of these mechanisms, there are also cases described where both mechanisms contribute to the resistant phenotype.[19]

As mentioned earlier, exploring the compatibility between BCAs and pesticides is key to developing effective and sustainable pest management strategies, essential for environmental resilience and food security.[20] Research is currently focused on developing a new generation of more selective pesticides and assessing the side effects of existing pesticides on natural enemies to achieve this compatibility.[21–23] In the same line of research, we have previously reported high levels of resistance to pyrethroids in a commercially sourced colony of *Phytoseiulus persimilis* Athias Henriot (Mesostigmata: Phytoseiidae).[24] Additionally, other authors have also described resistance to pesticides in several species of BCAs,[25–27] and there are reports of some phytoseiids exhibiting higher tolerance to pesticides than their prey.[28] However, in most agricultural scenarios, the integration of BCAs with the use of pesticides in an IPM context is still problematic. This is especially true in the case of pesticides, like pyrethroids, which are usually considered highly toxic for BCAs. In this study, we measured the susceptibility to deltamethrin in two colonies of *Amblyseius swirskii*, elucidated the determinants for the resistance detected, and assessed the possible compatibility of pyrethroid treatments with augmentative releases of this mite under field-like conditions.

2 MATERIAL AND METHODS

2.1 *Amblyseius swirskii* colonies

Two colonies were used in this study. The commercially sourced mites were purchased from Koppert Biological Systems, Berkel en Rodenrijs, The Netherlands (Swirski-Mite, 50 000 mites per bottle or Swirski-Mite Plus, 250 mites per sachet). These mites were used for the assays as soon as they arrived. They were not maintained in culture on our premises. The field-derived colony was established from mites collected in five locations across Israel and reared at IVIA (Instituto Valenciano de Investigaciones Agrarias, Montcada, València, Spain).[29] The mites were cultured in rearing units composed of a section of rigid black plastic placed on top of a water-saturated sponge.[30] Twice a week, they were provided with pollen of *Carpobrotus edulis* (L.) (Caryophyllales: Aizoaceae) for them to feed *ad libitum*.[31] The colony was maintained at 25 ± 2 °C, with a photoperiod of 16 h:8 h light/dark and 80% ± 10% relative humidity (RH).

2.2 Deltamethrin solutions

A 5 mg/mL stock solution of deltamethrin (45423; Sigma-Aldrich, St Louis, MO, USA) was prepared in 99.6% acetone (ACET-G0P-1K0; Labkem, Barcelona, Spain). This stock solution was further used to prepare the working solutions of deltamethrin used in the rest of the experiments. In these cases, 20% acetone or distilled water were used to dilute the stock. This is clearly stated in each case.

2.3 Bioassays

For both mite colonies, groups of ten mites per replicate (10 to 19 replicates) were treated with 20, 40, and 60 mg/L of deltamethrin (diluted from the stock with 20% acetone). The field rate was estimated at 12.5 mg/L according to the datasheet of Decis® Protech 10 mL (80269285; Bayer CropScience, Leverkusen, Germany). Control mites were treated with 20% acetone. The mites were collected using a fine paintbrush and placed on black filter paper (1457; Filter-Lab). Then, 2 µL of deltamethrin relevant concentration were applied on top of each mite using an automatic micropipette. Next, it was placed in a 20 mL borosilicate vial containing a moist piece of black filter paper (30 µL of distilled water). To feed the mites, pollen from *Carpobrotus edulis* was added *ad libitum* to each vial before closing.[31] The vials were left undisturbed at 25 ± 2 °C, 16 h:8 h light/dark, for 24 h. After this period, the mortality was assessed (a mite was considered dead if no movement was detected after probing with a fine paintbrush), and the live and dead mites were placed in separate vials and stored at −20 °C. The mortality was corrected using the Schneider-Orelli's formula.[32]

2.4 Analysis of sequences encoding domains II and III of *Amblyseius swirskii* VGSC

Genomic DNA was extracted from pools of 10 to 20 mites (Table 1) using DNAzol® (Thermo Fisher Scientific, Waltham, MA, USA) following the manufacturer's protocol. The relevant regions of domain II (residues 884 to 1013) and domain III (residues 1492 to 1801) (*Musca domestica* numbering) were polymerase chain reaction (PCR) amplified with the following primer combinations: for domain II, primers were 1F_IIS5-6 and 1R_IIS5-6; and for domain III, 1F_IIIS6 and 1R_IIIS6 (Table 2). For each amplification, the reaction mixture contained 0.4 µM of each primer, NZYTaq II 2× Green Master Mix (NZYtech, Lisbon, Portugal) (12.5 µL), and genomic DNA (1 µL) in a final volume of 25 µL. Cycling conditions were: 95 °C for 1 min, followed by 35 cycles of 95 °C for 20 s, 60 °C

Table 1. Number of mites on each pool

Colony	[Deltamethrin] (mg/L)	Phenotype	Number of mites in pool
Koppert	0	Alive	20
	40	Dead	12
	40	Alive	20
	60	Dead	10
	60	Alive	20
Field derived	0	Alive	20
	40	Dead	13
	40	Alive	20
	60	Dead	17
	60	Alive	20

Table 2. Oligonucleotides used to amplify and sequence *Amblyseius swirskii* voltage-gated sodium channel (VGSC) polymerase chain reaction (PCR) fragments

Name	(Sequence 5′ → 3′)
1F_IIS5-6	GCTTAGAAGGCGTCCAAGGA
2F_IIS5-6	CGTCCAAGGATTGTCGGTGT
3F_IIS5-6	CTCACTCTCGTTCTGTGCCA
1R_IIS5-6	AGGTTGCCAATAACGACGGT
1F_IIIS6	CAAGTGGCCACGTTCAAAGG
2F_IIIS6	GCCACGTTCAAAGGTTGGAC
1R_IIIS6	GTTTCGTCCATGATCGCAGC

for 20 s and 72 °C for 2 min, and a final extension at 72 °C for 10 min. The PCR fragments were purified with NucleoSpin® Gel and PCR Clean-up (Macherey-Nagel GmbH & Co. KG) and directly sequenced (STAB VIDA, Caparica, Portugal) with primers 2F_IIS5-6 for Domain II and 2F_IIIS6 for Domain III (Table 2). Further sequencing was carried out with the inner primer 3F_IIS5-6 when needed. All primers were designed, and the sequences were analyzed using Geneious software (version 10.2.6, http://www.geneious.com/).

The sequencing was carried out using pools of at least ten mites collected randomly from both colonies. Furthermore, pools of bioassayed mites were also sequenced. As stated earlier, they were separated, alive or dead, after the treatments.

2.5 Bioassays with synergists

Solutions of each synergist were prepared in 99.6% acetone (ACET-G0P-1 K0; Labkem) at the following working concentrations: 1% iprobenfos (IBP) (45814; Sigma-Aldrich), 0.5% diethyl maleate (DEM) (W505005; Sigma-Aldrich)[33] and 75 µg/mL piperonyl butoxide (PBO) (291102; Sigma-Aldrich).[34] Each mite was treated with 2 µL of the relevant synergist solution as described earlier. Then, they were placed on a sample vial, and incubated in groups of 40 mites for 1 h. After incubation, the mites recovered from the sample vials were treated as previously described with 20 mg/L deltamethrin (diluted from the stock with 20% acetone). The vials were left undisturbed at 25 ± 2 °C, 16 h:8 h light/dark, for 24 h. After this period, the mortality was assessed as described earlier.

2.6 Semi-field assays

Semi-field trials were conducted in a glasshouse facility at IVIA. A total of 75 sweet pepper plants (*Capsicum annuum*), approximately 40 cm in height, were used. Mites at all developmental stages were released using one sachet of Swirski Ulti-Mite (250 mites per sachet, Koppert Biological Systems) per plant. The plants were distributed in the glasshouse in 15 blocks of five plants each.

After 2 weeks, when the mites colonized the plants, we removed the sachets and conducted a mite count on 25 plants. Subsequently, using a 500 mL plastic manual sprayer, treatments with deltamethrin at 12.5 and 40 mg/L (diluted from the stock with distilled water), Decis Protech® at 12.5 mg/L, dimethoate at 600 mg/L (Perfekthion® at 0.1%) as a positive control, and a control treatment with distilled water were applied from 50 cm distance to achieve complete leaf wetting. Each treatment was applied to ten plants, treating 50 plants in total. As a wetting agent, all treatments contained 0.1% volume of OmniPur® Triton® X-100.

Mite counts after the treatments were conducted at 2 and 7 days under a stereomicroscope, recording the number of live and dead mites found. These counts were carried out exhaustively, involving the destruction of the plant.

2.7 Effect of persistent exposure to deltamethrin

Tests were conducted using modified Huffaker cells to assess the effect on mites of continuous and sustained contact with deltamethrin-treated leaf surfaces.[35–37] The experimental unit, a Huffaker cell, consists of three pieces of transparent acrylic: two measuring 80 mm × 40 mm × 5 mm and one measuring 80 mm × 40 mm × 10 mm with a central hole 2 cm in diameter. The three pieces were joined together, leaving the one with the hole in the middle and held together with elastic bands.[37] Three different treatments are applied: 40 mg/L deltamethrin (diluted from the stock with distilled water), dimethoate (Perfekthion® 0.1%, RSCO-INAC-0124-017-009-037; BASF, Ludwigshafen, Germany) and a negative control treatment of distilled water. All treatments contained 0.1% (*v/v*) OmniPur® Triton® X-100 (8787; Merck KGaA, Darmstadt, Germany) as a wetting agent. Pepper plants, 30 cm in height, were uniformly sprayed using a 500 mL plastic hand sprayer until they were completely wet. Then, they were allowed to air dry. After drying, the leaves were used to cut out 2 cm leaf discs using a metal cylinder. These discs were then placed in the central cell of each Huffaker cell. Ten mites per replicate were added to the discs, and *Carpobrotus edulis* pollen was used as a food source. Five technical replicates were carried out for each treatment.

The cells were incubated at 25 ± 2 °C, 60–65% RH, and a 16 h:8 h light/dark photoperiod. Mortality was assessed after 48 h under a stereomicroscope (a mite was considered dead if no movement was detected after probing with a fine paintbrush). The data were normalized using the Schneider-Orelli formula.[32]

2.8 Evaluation of the olfactive response

These experiments were conducted using a Y-tube olfactometer that consisted of two 5 L volume jars connected with a 2.4 cm diameter Y-shaped glass tube with a 13.5 cm long base and two arms of 5.75 cm long each.[38] Both side arms were connected via high-density polyethylene (HDPE) tubes to the two identical glass jars. Each glass jar was connected to an air pump that produced a unidirectional humidified airflow at 150 mL/min. Four 60 cm fluorescent lamps (L18 W/765; OSRAM GmbH, Munich, Germany) were positioned 40 cm above the horizontal Y-shaped glass tube. The light intensity registered over the Y-tube was 2.516 lx, measured using a ceptometer (LP-80 AccuPAR; Decagon Devices, Inc., Pullman, WA, USA). The Y-tube experiments were conducted under the following environmental conditions: 23 ± 2 °C and 60% ± 10% RH. Small pepper plants were treated 24 h before starting the assays with the Y-tube. The size of the plants was small enough for them to fit inside the jars without suffering any damage that may jeopardize the measurement quality due to damage-related volatile release. The plants were treated as described earlier with 40 mg/L deltamethrin (diluted from the stock with distilled water) supplemented with 0.1% OmniPur® Triton® X-100 as wetting agent. The control treatment was distilled water supplemented with 0.1% OmniPur® Triton® X-100. To verify that the olfactometer was functioning properly, 40 female specimens were selected under a stereomicroscope and first passed through both empty jars, with no preference observed. Then, a

plant treated with 40 mg/L deltamethrin was placed in one jar and treated with the control solution in the other jar. Each of the tested females of *Amblyseius swirskii* ($n = 40$) was placed individually at the entrance of the Y-tube. For every five mites, the Y-tube was rotated to limit external effects; for every ten mites, the Y-tube and the guide were cleaned with 99.6% acetone, and for every 20 mites, the plants in the jars were changed. A response is considered when the mite spends more than 5 s on one side; a non-response is considered if more than 5 min have passed, and the mite has not reached the chosen side and was excluded for data analysis. The time used to obtain the response was also measured.

2.9 Repellency and irritancy bioassays

The methodology was adapted from Beers and Schmidt-Jeffris.[39] The bioassay arena consisted of a pepper leaf disc 4 cm in diameter. The disc was cut so that it was bisected by the midvein, which served as the division between the treated and untreated halves. One of the disc halves was treated by dipping it for 3 s in the test solution; the other half was left untreated. The test solutions were 12.5 mg/L deltamethrin (diluted from the stock with distilled water) or distilled water. In both cases 0.1% OmniPur® Triton® X-100 was used as wetting agent. These discs were allowed to air dry for 1 h, and then they were placed in 55 mm Petri dishes. Ten *Amblyseius swirskii* females were selected under a stereomicroscope and transferred to each disc, placing them on the midvein to minimize bias. The mites were left undisturbed for 1 h, and then we evaluated the number of mites on each half as well as the number of mites leaving the arena (run-off).

2.10 Repellency of treated plants

A new semi-field trial was conducted to quantify the possible repellency effect of deltamethrin over *Amblyseius swirskii*. Mites at all developmental stages were introduced to each plant using one sachet of *Swirski Ulti-Mite* (containing 250 mites per sachet, Koppert Biological Systems). The sachet was placed 2 weeks prior to applying treatments to allow the mites to colonize the plant following the same protocol as in section 2.6. This experiment was conducted in a glasshouse facility at IVIA. The pepper plants, approximately 40 cm in height, were treated as previously described (section 2.6) with either 12.5 mg/L deltamethrin (diluted from the stock with distilled water) or distilled water (control). Both treatments contained 0.1% of OmniPur® Triton® X-100 as a wetting agent. A total of eight biological replicates were carried out for each treatment. A sticky trap (Tangle Trap®, Scotts Miracle-Gro, Marysville, OH, USA) was placed at the base of each plant to catch mites escaping from the plant. After 7 days, the number of mites in the trap and on the plant was recorded exhaustively, involving the destruction of the plant. The experimental unit consisted of individual plants randomly arranged in four blocks, replicated four times each.

2.11 Statistical analyses

All statistical analyses were conducted using GraphPad Prism version 9.00 for Windows (GraphPad Software, La Jolla, CA, USA, www.graphpad.com). To compare susceptibility to deltamethrin across different colonies, we used the Mann–Whitney test, which is suitable for non-parametric data with independent groups. Before running the Mann–Whitney test, data were assessed for homoscedasticity and normality to confirm the suitability of non-parametric methods for analysis. The effect of the plant treatments on the abundance of mites was analyzed using a one-way analysis of variance (ANOVA), followed by a *post hoc* Bonferroni's multiple comparisons test to assess the differences among treatments. Olfactometer data were analyzed using Pearson's χ^2 test to examine distribution differences, while results from the disc arena assays were analyzed with the Mann–Whitney test. Additionally, repellency and irritancy responses were compared using the Mann–Whitney test to account for non-parametric distributions.

3 RESULTS

3.1 Susceptibility to deltamethrin

Mites from both colonies were bioassayed using varying concentrations of deltamethrin to assess their susceptibility to this pyrethroid. The mites from Koppert have lower susceptibility compared to those from the field-collected colony, showing 3% mortality at 20 mg/L, 5% mortality at 40 mg/L, and 6% mortality at 60 mg/L (Fig. 1). Conversely, mites from the field-collected colony showed significantly higher mortality when tested with the same concentrations. Mortality at 20 mg/L was 18% (Mann–Whitney $U = 23.50$, $P = 0.00478$), at 40 mg/L was 28% (Mann–Whitney $U = 35.00$, $P = 0.00004$), and at 60 mg/L, was 37% (Mann–Whitney $U = 15.00$, $P = 0.00037$) (Fig. 1). The average mortality in the control, non-treated groups, was 6.7% in the Koppert colony (not significantly different from that of the tested group, Mann–Whitney $U = 112.5$ $P = 0.5256$ at 60 mg/L) and 6.9% in the field-collected colony ($P < 0.001$) However, despite the disparities in mortality rates between colonies, numerous mites within the field-collected colony were able to survive when exposed to concentrations nearly five times higher (60 mg/L) than the maximum rate recommended for field application (12.5 mg/L).

3.2 Variations in the sequence of the VGSC gene between colonies

The sequence of relevant genomic regions of domains II and III of the VGSC revealed variations between the two colonies that might impact their susceptibility to deltamethrin. The differences

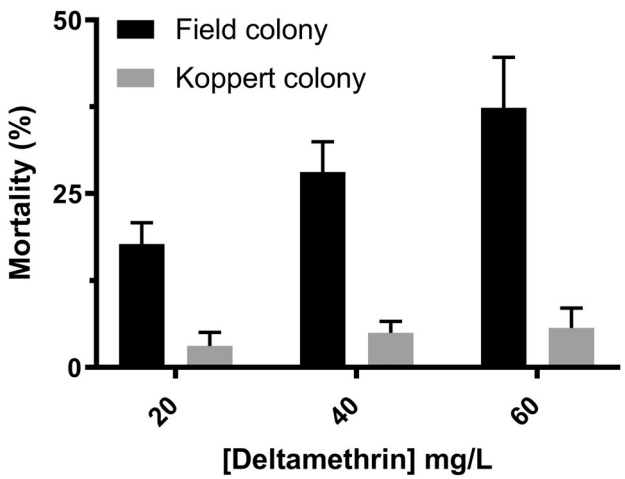

Figure 1. Susceptibility to treatments with deltamethrin in the two *Amblyseius swirskii* colonies. The bars represent the normalized mortality[32] of each treatment. Error bars represent the standard error of the mean (SEM). Asterisks on top of the bars indicate statistically significant differences, with the number of asterisks indicating the level of significance: $^{**}P \leq 0.01$, $^{***}P \leq 0.001$, and $^{****}P \leq 0.0001$.

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in domain II were located at positions 918 and 925 of the channel protein (*Musca domestica* numbering). The pool of mites from Koppert was homogeneous in the genetic regions analyzed, having methionine at position 918 and valine at position 925 (Fig. 2). However, the pool of mites from the field-collected colony showed a more heterogeneous pattern, having either methionine or leucine at position 918 and leucine or valine at position 925 (Fig. 2). There were no differences in domain III between the two sets of mites (Supporting Information Fig. S1).

3.3 VGSC sequence analysis of bioassayed mites

As expected, given the homogeneous patterns observed when testing random mites from the Koppert colony (Fig. 2), the sequences obtained from mites were the same for the mites surviving or dying after the treatment with deltamethrin. They all have single peaks in the electropherogram, indicating methionine at position 918 and valine at position 925 (Fig. S2). It is important to point out that, in this case, there is no significant difference between the number of mites dying after treatment and those dying in the non-treated controls.

However, the patterns obtained from bioassayed field collected mites were again heterogeneous. The sequences obtained from the pool of mites killed with 40 mg/L of deltamethrin showed a 'clean' electropherogram, indicating that all of them have methionine at position 918 and leucine at position 925 (Fig. 3). The data from the mites surviving the treatment at the same concentration showed that there was a mixture of genotypes among them, making it impossible to say whether they were homozygous for methionine or leucine at position 918 and/or for leucine or valine at position 925 or if they were heterozygous (Fig. 3). Similar mixed patterns were observed in the data obtained from both live and dead mites treated with 60 mg/L (Fig. 3).

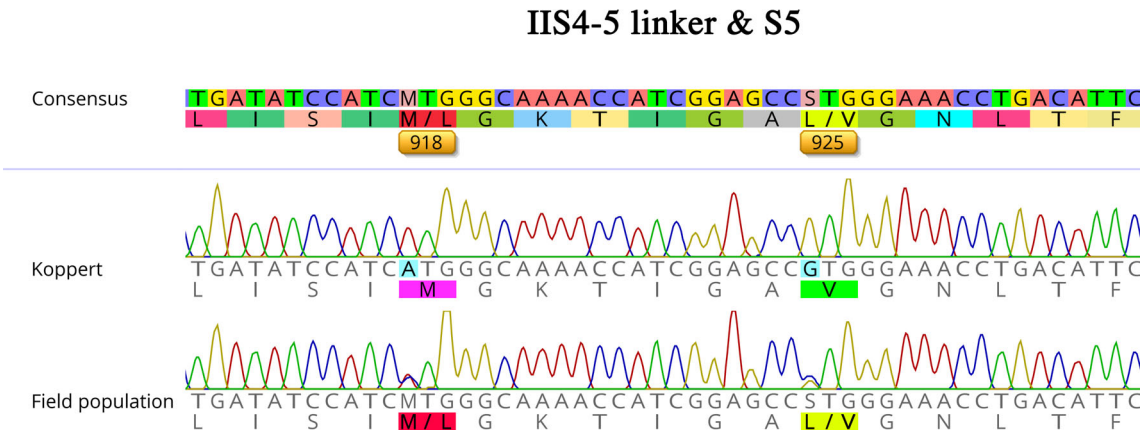

Figure 2. Genetic variability in relevant regions of the VGSC. Electropherograms obtained after sequencing DNA extracted from a pool of at least ten mites from each colony. The region shown corresponds to the partial sequence of the linker between transmembrane segments 4 and 5 (residues 914 to 919) and segment 5 (residues 923 to 930) of domain II. At the top is the consensus sequence for both colonies, with the differential residues highlighted.

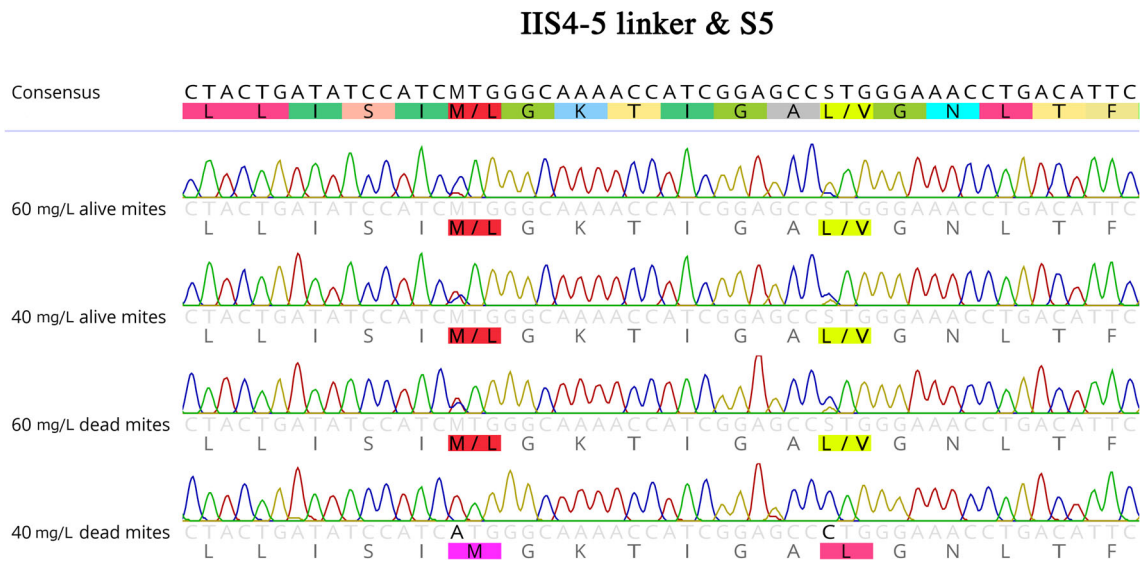

Figure 3. Polymorphisms on live and dead bioassayed mites from field-derived colony. Genotypic profiles of pools of mites from a field-derived colony according to their susceptibility to treatments with deltamethrin (40 and 60 mg/L). The partial sequence of the region corresponding to the linker between IIS4 and IIS5 (residues 914 to 919) and IIS5 (residues 923 to 930) of the VGSC is shown. The highlighted residues in the chromatograms correspond to those where variability is found among the samples analyzed.

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3.4 Effect of synergists over the susceptibility to pyrethroids

Similar bioassays were conducted to assess whether the observed reduced susceptibility to deltamethrin in the mites from Koppert was influenced by the overexpression of one or multiple detoxification enzymes. In this case, the mites were pre-treated with synergistic compounds before the treatment with deltamethrin. Notably, pre-treatment with PBO led to a significant five-fold increase in the mortality of mites when they were further treated with 20 mg/L deltamethrin (Mann–Whitney $U = 13.50$; $P = 0.0137$) (Fig. 4). This increase in the toxicity of deltamethrin after the treatment with PBO indicates that overexpression of one or more cytochromes P450s may be contributing to the resistant phenotype. Pre-treatments with DEM or IBP did not lead to any significant variation in the toxicity of deltamethrin, so it is unlikely that glutathione-S-transferases or carboxylesterases are contributing to the resistant phenotype, respectively. (Fig. 4).

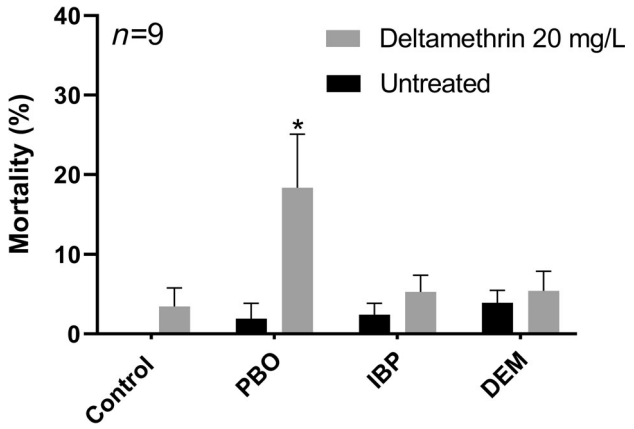

Figure 4. Impact of synergistic compounds on the toxicity of deltamethrin. The bars depict the normalized mortality of *Amblyseius swirskii* mites from Koppert pre-treated with synergistic compounds (PBO, IBP, DEM) and treated afterwards with 20 mg/L deltamethrin. Error bars represent the standard error of the mean (SEM). Asterisks on top of the bars indicate statistically significant differences, with the number of asterisks indicating the level of significance: *$P \leq 0.05$.

3.5 Semi-field assays

To assess the effect of deltamethrin in field-like conditions, the mites were released on pepper plants that were later treated with either Decis Protech® at 12.5 mg/L or deltamethrin at 12.5 and 40 mg/L. Initial mite counts (before treatment) averaged 93.88 mites/plant, referred to as untreated plants in Fig. 5. Two days following the application of Decis Protech® at 12.5 mg/L, mite counts dropped to 10.6 mites/plant, decreasing further to 1 mite/plant after 7 days. Similar trends were observed with deltamethrin treatments, where mite counts reached 20 mites/plant 2 days after treatment with 12.5 mg/L and 3.6 mites/plant after the treatment with 40 mg/L. Seven days post-treatment, the averages were 2 mites/plant for the 12.5 mg/L treatment and 0.2 mites/plant for the 40 mg/L treatment. Control treatment exhibited mite counts of 83 mites/plant at 2 days and 81.4 mites/plant at 7 days. The treatment with 600 mg/L of dimethoate drastically reduced mite counts to 0.6 mites/plant at both intervals (Fig. 5). Statistical analysis of data showed that the differences were significant at two ($F_{4,20} = 19.65$; $P < 0.0001$) as well as at 7 days ($F_{4,20} = 24.95$; $P < 0.0001$). The *post hoc* Bonferroni's multiple comparisons test revealed significant differences among all treatments compared to the control at 2 days ($P < 0.0001$) and 7 days ($P < 0.0001$).

Interestingly, we have detected a significant reduction in the number of live mites in all treatments comparing control plants. However, we have only detected dead mites in the plants treated with 600 mg/L of dimethoate.

3.6 Effect of continuous exposure to deltamethrin

The impact of prolonged mite contact with treated leaf surfaces was examined through bioassays using Huffaker cells (Fig. 6). In these bioassays, the corrected mortality of mites after the treatment with deltamethrin at 40 mg/L for 48 h was 5.2%. In contrast, the treatment with Perfekthion® 0.1% (*v/v*) (dimethoate 600 mg/L) induced 100% mortality at 48 h. Mortality rates in the non-treated control were 2.5% after 48 h (Fig. 6), not statistically different from that of the treatment with 40 mg/L deltamethrin (Kruskal–Wallis $Z = 0$; $P \geq 0.9999$).

3.7 Evaluation of the olfactory response

To assess if the treated plants are releasing any volatile that might be affecting the behavior of mites, the olfactory response of

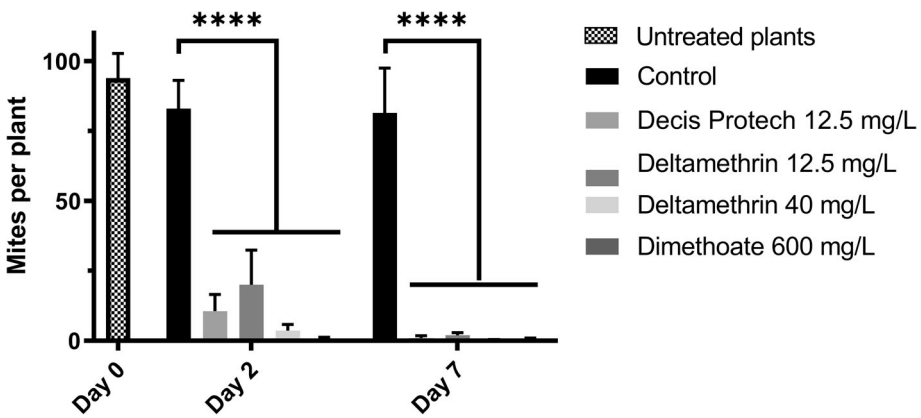

Figure 5. Abundance of mites on semi-field trials. Mite counts per plant in the different treatments at 2 and 7 days. The mite count before applying any treatment at Day 0 is also shown (checkered pattern column). Error bars represent the standard error of the mean (SEM). The treatments are grouped into statistically significant clusters. Asterisks on top of the bars indicate statistically significant differences, with the number of asterisks indicating the level of significance: ****$P \leq 0.0001$.

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female mites to plants treated with deltamethrin was assessed using a Y-tube olfactometer. In the assays, the mites did not show any statistically significant attraction nor repellency to the deltamethrin-treated plants in comparison with those untreated; 55% of the mites chose the untreated plant, while 45% chose the treated plant (Pearson's χ^2 test, $P = 0.371$) (Fig. 7). The control assay showed a uniform distribution in the choice of odor source, confirming that there are no external factors influencing the results.

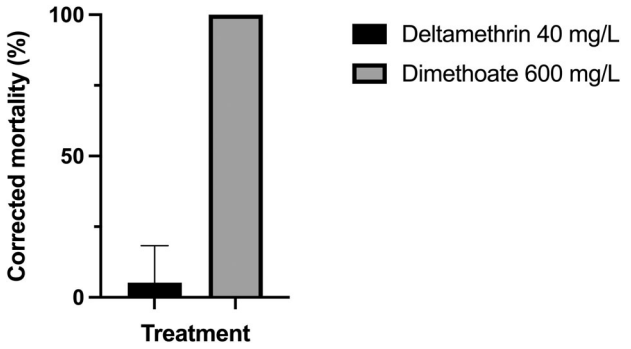

Figure 6. Effect of continuous exposure to deltamethrin. The bars represent normalized mortality for treatments with 40 mg/L deltamethrin or 600 mg/L dimethoate (Perfekthion® 0.1%) for 48 h. Error bars depict the standard error of the mean (SEM).

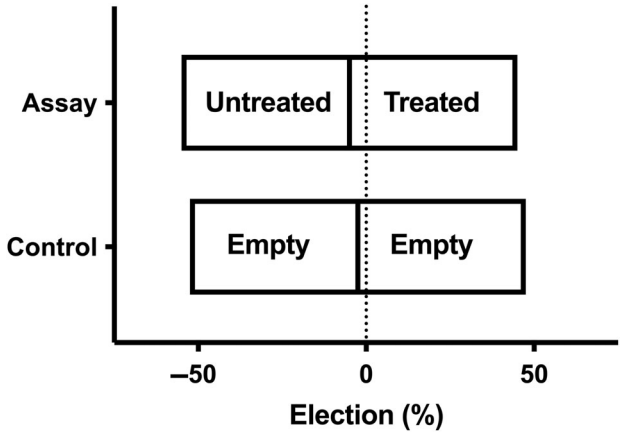

Figure 7. Olfactory response to treated plants. Results obtained in olfactory response assays. The percentage of mites responding to olfactory stimuli are shown. The top part represents the response of untreated plants *versus* treated plants, and the bottom part shows the results without using any volatile source.

3.8 Repellency and irritancy bioassays

The effect of surfaces treated with deltamethrin was assessed through choice assays (Fig. 8). The mites showed no different preference for remaining on the leaf disc halves treated with 12.5 mg/L deltamethrin or those treated with water (Mann–Whitney $U = 1259$; $P = 0.7818$). However, there were significant differences in the number of mites leaving the arena (run-offs). Here, 55.88% of mites left the arena when treated with deltamethrin, while only 43.73% left it when treated with water (Mann–Whitney $U = 942.5$; $P = 0.0154$) (Fig. 8).

3.9 Repellency of treated plants

A semi-field assay was designed to assess whether the mites run away from the plants when treated with the pesticide. The data collected in these trials indicated that, after 7 days, there was a significant difference in the number of mites found on the plants treated with deltamethrin when compared to control plants treated with water (Fig. 9) (Mann–Whitney $U = 0$; $P = 0.00002$). In close relationship with this, the number of mites stuck to the traps on the stem of plants treated with deltamethrin at 12.5 mg/L was significantly higher than those found on untreated plants (Fig. 9) (Mann–Whitney $U = 13$; $P = 0.0462$).

4 DISCUSSION

The compatibility between BCAs and pesticide applications in agricultural settings is becoming increasingly important, particularly within the framework of IPM strategies. The overarching aim of numerous regulatory bodies, spearheaded by the European Union, is to control pest populations in crops either without or with minimal reliance on pesticides.[40] Integrating biological control approaches with judicious pesticide application is a pivotal strategy to reach this objective. Here, we assess the impact of deltamethrin treatments on the predatory mite *Amblyseius*

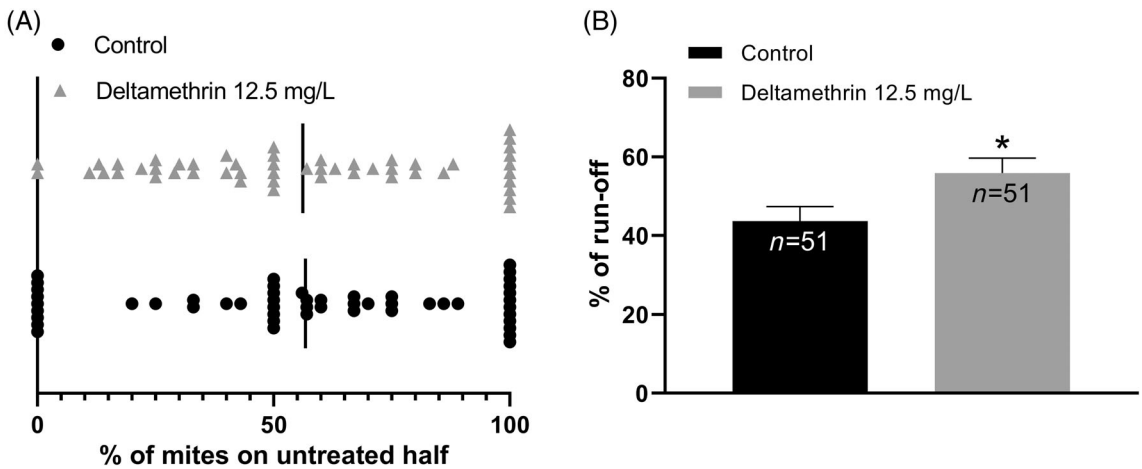

Figure 8. Repellency of deltamethrin to *Amblyseius swirskii*. (A) Percentage of mites found on the untreated half of the leaf disc relative to the total mites found on the leaf disc. (B) Percentage of total mites that are not on the leaf disc, that is, escaped from the leaf disc. Asterisks on top of the bars indicate statistically significant differences, with the number of asterisks indicating the level of significance: *$P \leq 0.05$.

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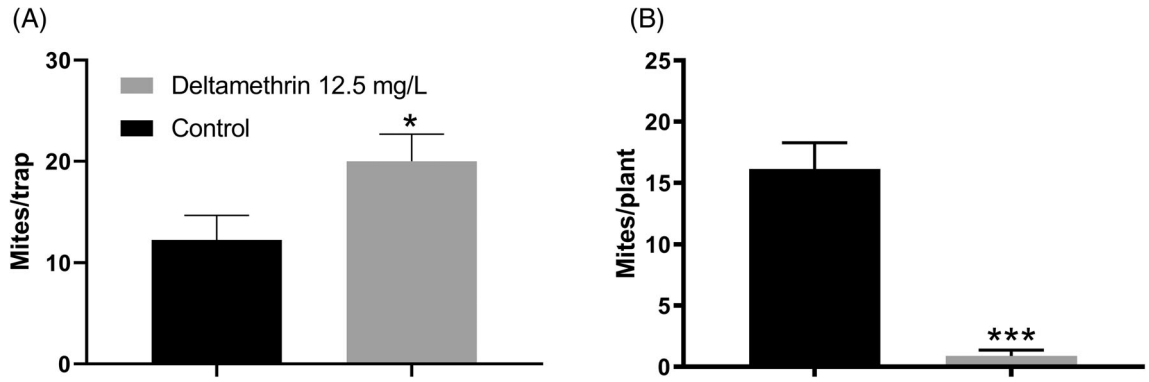

Figure 9. Results of repellency in treated plants. (A) Mean number of mites found in each trap for the different treatments. (B) Mean number of mites found per plant for each treatment. Asterisks on top of the bars indicate statistically significant differences, with the number of asterisks indicating the level of significance: *$P \leq 0.05$ and ***$P \leq 0.001$.

swirskii, a highly effective and extensively utilized predatory mite.[8–10,41]

In the laboratory bioassays, many mites from both colonies survived after treatment with deltamethrin at concentrations up to five times the recommended maximum field rate. However, there were evident differences between the colonies. The colony obtained from Koppert displayed the lowest mortality rates, akin to those observed in the control group of non-treated mites. Conversely, the colony sourced from field-collected mites exhibited slightly higher mortality rates, albeit still substantially low, considering the concentrations used in the bioassays. These discrepancies in susceptibility may be attributed to genetic differences within the colonies. A previous study assessing the microsatellite frequency in mites from the same sources revealed that those sourced from Koppert exhibited significantly lower heterogeneity than those collected from the field.[29] This discrepancy is expected, given that the mites from Koppert underwent many generations of mass-rearing in controlled conditions following field collection. Our data further aligns with this observation, as sequences obtained from relevant regions of the VGSC, the target site of pyrethroids, showed homogeneity in mites sourced from Koppert and heterogeneity in recently field-collected mites.

The sequences mentioned earlier were obtained to assess whether the phenotypic differences observed can be correlated with the presence of polymorphisms in the binding site of pyrethroids located in specific regions of the VGSC. The mites from Koppert were homogeneous for methionine at position 918 and valine at position 925 of the channel protein (*Musca domestica* numbering). We also found no genetic differences between the mites that survived the treatments and those that did not (Fig. S2). However, the mites from the field-collected colony were heterogeneous, they have methionine and/or leucine at position 918 and leucine and/or valine at position 925. Since we sequenced pools of mites, it is impossible to say whether they were homozygous or heterozygous. In this colony, the genotyping results of bioassayed mites are somewhat more challenging to interpret. Except for the mites that die at a concentration of 40 mg/L, which show only the alleles for methionine at position 918 and leucine at position 925, the rest of the pools analyzed after the bioassays show a mixture of genotypes. Despite this, in *Phytoseiulus persimilis*, we previously observed that the L925V mutation consistently segregates between surviving and dead mites when bioassayed with 40 mg/L deltamethrin.[24] For this reason, we hypothesize that the most plausible explanation is the contribution of other resistance mechanisms to the observed phenotype in the field-collected colony.

Residues at positions 918 and 925 are remarkably conserved in arthropods and crucial for the interaction of pyrethroids with their binding site,[42–44] with methionine and leucine present in wild-type individuals, respectively.[18] Extensive literature reviews the impact of amino acid variations at these two positions, but these variations are always associated with a certain level of resistance to pyrethroids.[18] This is because, in modeling studies it was proposed that they form part of a hydrophobic pocket that accommodates the pyrethroid when interacting with the channel.[43,44] Any variation in the pocket structure may be impairing the capacity of the pyrethroid to bind properly, hence a direct cause for loss of toxicity. In fact, the mutations found in this study, M918L and L925V, have been described previously as associated with resistance to pyrethroids in the predatory mite *Phytoseiulus persimilis*.[24] Also, in *Neoseilus barkeri* Hughes, 1948 (Mesostigmata: Phytoseiidae) mutation M918L was uncovered through data mining.[27] Additionally, these mutations have been described in other arthropods such as *Aphis gossypii* (Glover) (Hemiptera: Aphididae),[45] *Myzus persicae* (Sulzer) (Hemiptera: Aphididae),[46] *Hyalella azteca* (Saussure) (Amphipoda: Hyalellidae),[47] *Trialeurodes vaporariorum* Westwood (Hemiptera: Aleyrodidae),[48] *Thrips tabaci* Lindeman (Thysanoptera: Thripidae),[49] *Varroa destructor* Anderson & Trueman (Mesostigmata: Varroidae),[50,51] *Rhopalosiphum padi* (Linnaeus, 1758) (Hemiptera: Aphididae),[52] *Dermanyssus gallinae* (De Geer, 1778) (Mesostigmata: Dermanyssidae),[53,54] *Sarcoptes scabiei* var. *hominis* (Astigmata: Sarcoptidae)[55] and *Hyalella azteca* (Amphipoda: Hyalellidae).[56]

The level of genetic homogeneity observed in the mites sourced from Koppert is intriguing, especially considering that all mites bore a mutation associated with resistance despite purportedly lacking prior exposure to pyrethroids. Typically, such a scenario might be expected in mites subjected to stringent selection regimes in laboratory conditions or in colonies derived from mites repeatedly exposed to field sprays. This last explanation is the most plausible scenario to explain the fixation of the L925V mutation in the Koppert population. However, it appears evident that resistant alleles are spread among populations since they were also detected in mites from the other colonies studied in this work. This second colony originated from five samples collected in Israel and subsequently reared together in laboratory

conditions with no pesticide exposure after collection.[29] These alleles indicate that at least one of the original populations must have been exposed to pyrethroids. This exposure would have been selected for individuals carrying alleles that confer lower susceptibility, including mutations in the VGSC gene, but potentially other resistant alleles.

The presence of individuals bearing mutations associated with resistance is a straightforward explanation for the low susceptibility to the treatment with deltamethrin in the colonies analyzed, but this was only part of the story. It is known that variations in the expression or regulation of detoxifying enzymes may also contribute to the resistant phenotype. The assays conducted using synergistic compounds carried out with the colony sourced by Koppert showed that part of these enzymes may be contributing to the resistance phenotype. The pre-treatment with PBO increased up to five times the mortality after the treatment with deltamethrin, evidencing the possible role of the cytochrome P450 family. A total of 77 coding unigenes for P450 monooxygenases from different clades (CYP2, CYP3, CYP4, and mitochondrial CYPs) were identified in a colony of *Amblyseius swirskii* from Koppert.[57] In comparison, *Galendromus occidentalis* (Nesbitt) (Mesostigmata: Phytoseiidae), another predatory mite, has 63 complete sequences for P450s, while *Tetranychus urticae* Koch (Trombidiformes: Tetranychidae), a phytophagous mite, had 86 CYP genes.[58,59] The association of P450 monooxygenases with pesticide resistance has been documented in many arthropod species.[60] For instance, in the pollen beetle *Brassicogethes aeneus* (Fabricius) (Coleoptera: Nitulidae), the overexpression of the enzyme CYP6BQ23 in populations resistant to pyrethroids demonstrated its efficient metabolism of deltamethrin.[61] In *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae), the enzymes CYP6AE43 and CYP6AE48 were identified as responsible for the detoxification of pyrethroids, and the silencing of these enzymes increased susceptibility to pyrethroids in the larvae.[62] In phytoseiid mites, a population of *Euseius citrifolius* Denmark & Muma (Mesostigmata: Phytoseiidae) resistant to abamectin showed the association of P450s with the resistance detected.[63] Also, an increase in P450s activity was detected in a laboratory-selected population of *Neoseiulus californicus* (McGregor) (Mesostigmata: Phytoseiidae) resistant to spiromesifen.[64] Thus, taking all our data together, the low susceptibility to pyrethroids detected in the bioassays is very likely associated with modifying the target site and increasing pesticide oxidation by monooxygenases cytochrome P450s.

Instances of BCAs developing resistance to pesticides through selective pressures or exhibiting a broad spectrum of natural variability in their susceptibility to various pesticides have been documented previously.[25] Moreover, historical records include experimental releases of selected pesticide-resistant BCAs.[65–67] Gathering this evidence altogether shows that there is actual potential to develop commercial colonies with particular phenotypes that can be conveniently used as part of IPM strategies, as is currently done with species of insect predators like *Orius laevigatus* (Fieber) (Hemiptera: Anthocoridae).[22,23] However, a quick search on the websites of companies sourcing *Amblyseius swirskii*, as well as other phytoseiids, showed that deltamethrin is considered very harmful for them (https://sideeffects.koppert.com/side-effects), an apparent contradiction with the data presented here so far.

The semi-field assay, a crucial bridge between laboratory and field conditions, was conducted to validate the applicability of the results from the controlled environment. The findings revealed a significant decrease in mite populations on the plant being sprayed with deltamethrin at field rates (12.5 mg/L) (Fig. 5). This contrasted with the results from the laboratory bioassays, where this concentration did not show a significant increase in mortality compared to control treatments.

Several hypotheses could explain this discrepancy, such as increased mortality from prolonged contact with treated surfaces, a repellent effect mediated by volatiles, or contact-mediated repellency of mites on treated surfaces. Various assays were conducted to elucidate the cause of this contrast between laboratory and semi-field results.

The long exposure of mites to deltamethrin-contaminated surfaces confirms the results from the laboratory. The mites sourced from Koppert showed very little mortality after being in contact with the pesticide for 48 h. Other studies with mites from the same source showed a harmful effect of the pesticide, with a mortality of 47.42% and a 100% oviposition reduction after 72 h of exposure.[68] Since no genetic or biochemical tests were carried out with those mites, it is not possible to properly assess the differences observed. Despite being from the same commercial provider, the genetic background between the two batches may differ.

The possibility of volatile-mediated repellence was tested using an olfactometer. This hypothesis was rejected as there was no detectable preference for plants sprayed with deltamethrin compared to the control treatment. This is the first evaluation of the olfactory response of phytoseiid mites to deltamethrin. A previous study tested the potential repellent effect of bifenthrin on *Amblyseius swirskii* using treated nets but also found no effect.[69]

Studies on other phytoseiid mites measured the response of *N. californicus* to volatiles emitted by corn plants infested with *Tetranychus urticae*. The results showed that this mite could not differentiate between infested and non-infested plants.[70] However, a response was found in tests with *N. californicus* and *Amblyseius andersoni* (Chant) (Mesostigmata: Phytoseiidae). These mites could differentiate apple branches infested with *Panonychus ulmi* (Koch) (Trombidiformes: Tetranychidae) from those not infested.[71]

The analysis of the repellency or irritancy of surfaces treated with deltamethrin revealed no preference for mites for either treated or untreated surfaces. However, a significant increase in mites escaping from arenas containing deltamethrin-treated leaves was observed. Similarly, when retesting the variation in the number of mites in treated plants but counting those escaping from the plants or trapping them with Tangle Trap®, we have detected that instead of dying, most mites were running away from the plants after treatment. These results align with findings from a study on *G. occidentalis* using the pyrethroid lambda-cyhalothrin, where no apparent repellent effect was noted, but a high percentage of mites were seen fleeing from lambda-cyhalothrin-treated leaf discs.[39] Similarly, repellence against treated surfaces has been observed with the pyrethroids lambda-cyhalothrin, bifenthrin, and fenpropathrin in *Phytoseiulus persimilis* populations using concentrations similar to those used in the field.[72] In pyrethroid-resistant *Anopheles gambiae* Giles (Diptera: Culicidae), deltamethrin induces similar surface irritancy effects, increasing mosquito escape behavior.[73] This run-off response is also documented for pyrethroid-treated surfaces in *Tetranychus urticae*.[74] Field studies on *Amblyseius andersoni* (Mesostigmata: Phytoseiidae) reported detrimental effects of the pyrethroid tau-fluvalinate, including an increased escape rate.[75] In an exciting research, Kakoki *et al.*[76] have shown that this behavior might be species-specific

with *Amblyseius eharai* (Mesostigmata: Phytoseiidae) and *Phytoseiulus persimilis* actively avoiding permethrin – and bifenthrin-treated surfaces. At the same time, the population of *Euseius sojaensis* (Ehara) (Mesostigmata: Phytoseiidae) declined heavily after pesticide application.[76]

Our research underscores the prevalence of pesticide resistance among arthropod species, including BCAs. Specifically, we showed evidence that commercially sourced *Amblyseius swirskii* harbors multiple mutations associated to both metabolic and target-site resistance, and at least some of these resistance alleles are also present in wild populations. However, the mere presence of these alleles may not be sufficient for the effective integration of pesticide use and BCAs within an IPM strategy. Our findings stress the importance of conducting comprehensive studies to assess the global impact of pesticides. This holistic approach is crucial in developing tailored solutions that ensure the optimal performance of both, chemical and biological control methods.

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CONFLICT OF INTEREST STATEMENT

The authors have no relevant financial or non-financial interests to disclose.

AUTHOR CONTRIBUTION

LB-A, AU, MP-H and JG-C conceived the ideas and designed the methodology. LB-A, MP-H, JC and MA-V performed the experiments. LB-A and JG-C analyzed the data. LB-A and JG-C wrote the manuscript. All authors reviewed and gave final approval for submission.

DATA AVAILABILITY STATEMENT

All the data that supports the findings of this study are available in the main document and in the Supporting Information of this article.

SUPPORTING INFORMATION

Supporting information may be found in the online version of this article.

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