Research Article

Received: 14 March 2024 Revised: 11 June 2024 Published online in Wiley Online Library: 12 July 2024

(wileyonlinelibrary.com) DOI 10.1002/ps.8271

Assessment of non-target toxicity of insecticides on *Ganaspis brasiliensis* (Ihering) in laboratory and field conditions

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Abstract

Background: G1 strain *Ganaspis brasiliensis* (Ihering) has been recently released in both Europe and America as a biological control agent of the spotted wing drosophila, *Drosophila suzukii* (Matsumura). In initial phases of classical biological control programs, it becomes imperative to evaluate the susceptibility of parasitoids to insecticides, to identify the best alternatives to adopt in an integrated pest management and organic perspective. In this study, we evaluated lethal and sublethal effects of topical application of five different insecticides classes: neonicotinoids, diamides, pyrethroids, organophosphates and spinosyns. Additionally, we tested residual toxicity in field trials in vineyards and sweet cherry orchards.

Results: Adult wasps' susceptibility to different insecticides' classes were consistent between laboratory and field. Spinosad exhibited the highest toxicity, with a median lethal concentration (LC_{50}) of 0.00372 of the maximum field dose, and the highest knock-down effect in field trials, causing 92.5 ± 5% of mortality at T_0. λ-cyhalothrin showed sublethal effects on both male and female insects' longevity when applied at LC_{30}. In field trials, deltamethrin showed the highest persistence, causing significant parasitoid mortality up to 14 days after treatment. Conversely, cyantraniliprole was the least toxic active ingredient according to both topical and residual bioassays, even though its residues caused mortality up to 7 days after the treatment in the field.

Conclusion: Our results indicate that spinosad and λ-cyhalothrin are highly toxic to *G. brasiliensis*, making them incompatible with classical biological control programs. Cyantraniliprole exhibited lower toxicity, and may be considered a selective pesticide for the integrated management of *D. suzukii*.

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

Keywords: biological control; sublethal effects; IPM; Spinosad; Cyantraniliprole; *Drosophila suzukii*

1 INTRODUCTION

The spotted-wing drosophila, *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae), currently stands as the most destructive pest of small fruits and cherries, leading to significant economic losses globally.[1] Farmer revenue losses are tied to the unsuitability of berries for the market due to the fly's preference for laying eggs on healthy and ripe fruits, where larvae develop causing direct and indirect damages on fruit.[2]

In response to *D. suzukii* infestation, growers often resort to the application of conventional and broad-spectrum insecticides sprayed according to calendar schedules.[3,4] While a long-term management solution is still elusive, integrated pest management (IPM) strategies strive to minimize chemical reliance, thereby enhancing the sustainability and cost-effectiveness of controlling *D. suzukii*.[5] Currently, the most promising approach

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involves importing larval parasitoids from the native regions of the pest, Southeastern and Eastern Asia, given the lack of effective indigenous natural enemies in the invaded areas.[6]

Foreign explorations for co-evolved parasitoids in Asia have demonstrated that the parasitism of *D. suzukii* is primarily supported by three larval parasitoid species: *Asobara japonica* Belokobylskij (Hymenoptera, Braconidae), *Ganaspis brasiliensis* (Ihering), and *Leptopilina japonica* Novković & Kimura (Hymenoptera: Figitidae). In South Korea, the parasitism rate of *D. suzukii* ranged from 0% to 17%, with the braconid species being the main control agent.[7] In Yunnan province, southwest China, the two figitid parasitoids together achieved substantial parasitism rates of up to 63%. In this area, *G. brasiliensis* is the primary parasitoid of the pest, achieving maximum parasitism rates of 40%.[8,9] In Japan *D. suzukii* populations are almost exclusively controlled by *G. brasiliensis*, with parasitism rates reaching 75.6%.[8]

These field surveys, along with quarantine tests conducted on collected parasitoids[10–12] set the basis for the classical biological control (CBC) of *D. suzukii*, identifying *G. brasiliensis* as the most suitable candidate for the CBC programs. Laboratory and semi-field studies proved that a specific genetic group of *G. brasiliensis*, namely G1[9,13] exhibits the highest host specificity, targeting exclusively L1 and L2 *D. suzukii* larvae within fruits.[8,14] These findings led to governmental approvals for area-wide releases of *G. brasiliensis* G1 as Biological Control Agent (BCA) in Italy[15] and in the United States.[16] Initial release attempts in northern Italy have demonstrated *G. brasiliensis*'s ability to disperse, overwinter, and specifically parasitize *D. suzukii*,[17] encouraging the continuation of the CBC project and the integration of this biological control agent into current IPM programs against *D. suzukii*. To promote a synergistic combination between biological and chemical control, it is therefore crucial to assess the influence of insecticides on *G. brasiliensis*.

The acute toxicity and sublethal effects of insecticides have been evaluated for numerous non-target biocontrol arthropods species, including predators[18–20] and parasitoids.[21–24] Over the past decades, a variety of agrochemical products have been tested and approved for *D. suzukii* control in invaded areas to suppress the pest and limit resistance development.[25] Chemical classes commonly utilized to suppress *D. suzukii* population include pyrethroids, carbamates, organophosphates, diamides, and, to a lesser extent, neonicotinoids,[5,26,27] while organic farming strongly relies on spinosyns.[28] The latter, together with diamide and phosmet have been proven lethal to all life stages of *D. suzukii*, including egg and larval instars.[29] Several chemical classes (neonicotinoids, organophosphates, pyrethroids, and spinosyns) have been proven highly toxic to the pest's pupal parasitoids of the genera *Trichopria* (Hymenoptera; Diapriidae) and *Pachycrepoideus* (Hymenoptera; Pteromalidae).[30,31] Spinosad-based insecticides utilized in organic farming have demonstrated particular toxicity to Hymenopterans[32] and showed high mortality to *Pachycrepoideus vindemiae* (Rondani) (Hymenoptera: Pteromalidae) when exposed to contaminated host puparia.[33]

While evidence suggests that biological control with pupal parasitoids is incompatible with chemical application, there are currently no ecotoxicology studies investigating the effects on *D. suzukii* larval parasitoids, although they are considered the most suitable antagonists of the pest in its native environment.[6] The application of insecticides has the potential to affect all beneficial arthropods, including parasitoids.[18,19,32,34,35] Such an impact poses a risk to the long-term crop protection they could provide. However, the presence of semi-natural habitats acting as reservoirs can mitigate such negative effects by facilitating field recolonization from the surrounding areas.[36–38] In landscapes characterized by widespread land dedicated to specialized farming activities, the intensive use of insecticides discourages the dispersal of parasitoids, thereby reducing recolonization processes. This is particularly evident in sweet cherry cultivation, where the number of insecticide applications against *D. suzukii* can reach up to eight per season, depending on pest abundance, cultivar susceptibility, and environmental factors.[39] Such a scenario is particularly unfavorable for inoculative CBC programs, especially at initial stages when chemical applications, combined with low density of the released population and the absence of natural reservoirs, could undermine the success of the entire intervention. A rational management of insecticide treatments that integrates with biological control strategy can provide both short- and long-term pest control benefits. To attain this objective, it is essential to achieve a thorough comprehension of the selectivity of the available pesticides. This understanding is crucial for assessing risks to beneficial organisms and allowing the selection of insecticides that preserve important natural enemies.[40]

In this study, we provide a first step for this process, assessing the lethal and sublethal effects of topical applications of five different classes of insecticide on *G. brasiliensis* G1 adults. Additionally, we present the result of field trials, where three selected products have been tested to evaluate the lethal effects of residual contact on one of the most cultivated crops in Italy, grapevine (*Vitis vinifera* L.)[41,42] and one of the most affected by *D. suzukii* damage, sweet cherry (*Prunus avium* L.).[26,43]

2 MATERIALS AND METHOD

2.1 Insects

The *D. suzukii* colony was established from multiple field collections of living adults occurring in 2020 and 2021 in Northeast Italy (Trento province). Insects were mass-reared in the laboratories of the Edmund Mach Foundation (FEM) in San Michele all'Adige (Trento, Italy) under controlled conditions with a photoperiod of 16:8 (L:D) at 24 ± 2 °C and 70 ± 10% relative humidity. Adult flies were placed in rearing cages (30 × 30 × 70 cm, Bugdorm BD4F3074, MegaView Science Co., Ltd, Taichung, Taiwan) and provided with water and an artificial cornmeal diet as substrate medium, which was replaced twice a week. The diet was prepared according to Dalton *et al.* (2011) with few modifications. Sugar (15 g), soybean flour (10 g), yeast flakes (17 g), cornmeal (71 g), and agar powder (6 g) were added to 1 L of boiling water. After cooking for 30 min, the mixture was allowed to cool to 60 °C. At this temperature propionic acid (10 mL) and vitamin fortification mixture (5 g) were added. The diet was then poured into sterile petri dishes (9 cm diameter) and refrigerated for later use.

The *G. brasiliensis* G1 colony was established from specimens received from CABI quarantine facilities (Delémont, Switzerland) in 2019, previously collected from their native environment (Tokyo, Japan) in field sampling that occurred from 2015 to 2017.[8] The insects were maintained in quarantine laboratories at FEM in controlled conditions and photoperiod of 16:8 (L:D) at 24 ± 2 °C and 70 ± 10% R.H. Parasitoids were reared according to the methodology described in Rossi-Stacconi *et al.* (2022).[44] Adults' emersion was monitored daily to isolate insects of known age.

In 2022, a sample of both flies and parasitoids were shipped to the laboratories of the University of Verona (Verona, Italy), where new colonies were established to conduct field trials. Rearing conditions and methodology were the same as described above.

Pest Management Science published by John Wiley & Sons Ltd on behalf of Society of Chemical Industry.

2.2 Insecticides

Insecticides have been chosen to represent a wide range of different chemical groups currently utilized to suppress *D. suzukii*: neonicotinoids, diamides, pyrethroids, organophosphates, and spinosyns.[3,26,27,45] For the toxicological bioassays carried out in laboratory conditions in 2021–2022, commercial formulations of cyantraniliprole (Benevia), acetamiprid (Epik), (λ)-cyhalothrin (Karate Zeon), spinosad (Laser), and phosmet (Spada) were tested. For the field trials carried out in 2022–2023, commercial formulation of cyantraniliprole (Exirel), deltamethrin (Meteor), and spinosad (Laser) were utilized. For field trials, the cyantraniliprole formulation, Exirel, was changed compared with the laboratory experiments to follow national regulations that authorized Exirel for both 2022 and 2023 season (Health Ministry, Registration no. 18 383 of May 25, 2023). Although formulation was different, active ingredient (a.i.) and concentrations were the same as in the previously tested product (Benevia). Phosmet has been banned in 2022 fruit growing season,[46] thus no further tests were conducted in the field. Deltamethrin has been selected for the field trials due to its frequent use by local farmers as an affordable pyrethroid-based product.[45] Label information of each pesticide's formulation is shown in Table 1.[47]

2.3 Baseline toxicity bioassays

Topical bioassays (direct spray) were conducted in the laboratories at FEM from December 2021 to May 2022. Insecticide exposure started immediately after the dilutions of the five commercial insecticides. For this, products were added to distilled water under a fume hood and stirred for 15 min to ensure homogenization. *Ganaspis brasiliensis* adults (5 ± 1 days-old) were placed in a vial tube using a mouth aspirator and anaesthetized through cold exposure for 2 min. Using a soft brush, they were gently moved to sterile Petri dishes (Ø = 9 cm). Dishes were sprayed using 3 mL of insecticide solution at a constant pressure of 55 kPa using the Potter spray tower (Burkard Manufacturing Co. Ltd, Hertfordshire, England), allowing a standard deposit of 6–6.5 mg/cm^2 of insecticide solution on the surface area. Control samples were sprayed with distilled water. Following spraying, the insects were immediately transferred to plastic Dutscher rearing tubes (Ø × h: 28.5 × 95 mm) containing a hydrated cellulose plug at the bottom soaked with 2 mL distilled water and honey applied to the inner face of the lid. Four concentrations per insecticide were tested for cyantraniliprole (250, 350, 500, 1400 mL/hL), acetamiprid (22, 110, 220, 1100 mL/hL), spinosad (0.25, 1, 1.75, 2.5 mL/hL), phosmet (10.78, 15, 15.85, 26.25 mL/hL), and λ-cyhalothrin (2.5, 4.43, 6.93, 25 mL/hL). Doses selection was based on a preliminary screening conducted at standard dilutions of the field rate (100%, 50%, 10%, 5%) followed by a preliminary probit analysis conducted with resulting data. Each concentration was replicated from 12 to 20 times (60 to 100 adults, respectively). The tested adults were distributed in a 1:1 female to male ratio. Mortality of treated wasps was assessed at 48 h after exposure. Moribund and dead wasps were combined and considered as dead. Moribund refers to parasitoids that were not able to hold on the bioassay vials, due to clear sign of toxicity such as leg twitching and partial paralysis.[35,48]

2.4 Sublethal toxicity on longevity

Sublethal effects on longevity were tested on individuals survived to the exposure to the estimated lethal concentration 5% and 30% (LC_5 and LC_{30}) resulting from the baseline toxicity assay. Spraying was conducted as described in the previous paragraph. Forty-eight hours after insecticide application, for every concentration tested (LC_5 and LC_{30}), 30 survived parasitoids per sex were transferred individually into Dutscher rearing tubes (Ø × h: 28.5 × 95 mm). A cellulose plug moistened with 2 mL of distilled water was placed at the bottom of each tube. A second cellulose plug, coated with a drop of honey on the inner side, was used to seal the vial and provide a food source. Water was added weekly to ensure that the bottom plugs were maintaining an adequate moisture level. Parasitoid mortality was monitored every other day and day of death was recorded.

Table 1. Label information on the pesticides tested in the laboratory toxicity bioassays and field trials

Active ingredient	Trade name and formulation*	Chemical group (Group code)†	Maximum label dose (mL/hL; a.i. g/L)	Target crop	Target pest	LAB‡	FIE§
Acetamiprid	Epik SL	Neonicotinoid (4A)	220; 50	Raspberry	*Drosophila suzukii*	X	
Cyantraniliprole	Benevia EC	Diamide (28)	75; 100	Strawberry	*Drosophila suzukii*	X	
Cyantraniliprole	Exirel SE	Diamide (28)	75; 100	Cherry	*Drosophila suzukii*		X
λ-cyhalothrin	Karate Zeon CS	Pyrethroid (3A)	20; 100	Stone fruit	*Drosophila suzukii*	X	
Deltamethrin	Meteor SC	Pyrethroid (3A)	80; 15.7	Cherry	*Drosophila suzukii*		X
Phosmet	Spada 200 EC	Organophosphate (1B)	375; 200	Stone fruit	*Ceratitis capitata*	X	
Spinosad	Laser SC	Spinosyn (5)	25; 480	Grapevine	*Drosophila suzukii*	X	X

*Formulations: Liquid Solution (LS), Emulsifiable Concentrate (EC), Suspo-Emulsion (SE), Capsule Suspension (CS), Suspension Concentrate (SC).
† Group code according to the Mode of Action (MoA) classification of the Insecticide Resistance Action Committee (IRAC).[47]
‡ Laboratory bioassays (LAB) conducted in 2021–2022.
§ Field trials (FIE) conducted in 2022–2023.

2.5 Toxicity of residual exposure in field trials

Field trials were conducted on grape in 2022 and on cherry in 2023. The tested insecticides were applied at maximum label dose (Table 1) using a motorized backpack sprayer (FOX MOTORI SRL, Poviglio, Italy) equipped with an air inclusion flat fan spray green nozzle AI110015VS (Teejet Technologies, Glendale Heights, USA) with an application volume of 1000 L/ha. The first trial was performed in a pergola-trained vineyard (cv. Corvina) located in the San Pietro in Cariano municipality (Verona, Italy) (45° 30′ 22.1″ N 10° 52′ 16.2″ E) and insecticide application was carried out on September 12, 2022 (T_0). Treatment plots consisted of 10 consecutive grapevines separated by untreated rows to prevent the effects of potential drift contamination. One hour after insecticide application, allowing time needed for vegetation to dry, parasitoids were placed in contact with the treated vegetation using a cylindrical net sleeve cage (diameter: 12 cm, length: 25 cm). Each cage, containing at least one treated leaf, housed ten 5 ± 2 days-old *G. brasiliensis* adults (five females and five males). Four net sleeves were installed per each treatment plot and untreated control (four replicates). Honey droplets and a water dispenser were inserted in the net sleeve to avoid insect death due to a lack of food/water resources. New clean net sleeves containing new insects were installed on plants 3, 7, 14, and 21 days after the application (September 15, 19, 26, and October 3, 2022). Seventy-two hours after each insect caging, caged vines were cut, brought to the laboratory, net sleeves removed, and the number of dead parasitoids was counted. Dataloggers (RC-51H, Elitech, London, UK) were installed inside the net sleeve to measure temperature and relative humidity conditions experienced by the parasitoids during the trials.

The trial conducted on cherry trees took place in a Kym green bush-trained orchard (cv. Ferrovia) located in the Grezzana municipality (Verona, Italy) (45° 33′ 06.1″ N 11° 02′ 46.7″ E), with insecticide application occurring on June 27, 2023 (T_0). Treatment plots consisted of four consecutive cherry plants separated by an untreated row. In the net sleeves, in addition to leaves, five treated cherries were enclosed. Mortality estimation was carried out 3, 7, and 14 days (June 30, July 4 and 11, 2023) after application. The evaluation was not extended further as cherries were no longer naturally available on the plant. The insecticide application methodology was as described above for the vineyard trials.

2.6 Statistical analysis

The baseline toxicity of five tested insecticides on *G. brasiliensis* was assessed using a probit regression model through a logarithmic transformation of the data[49] using SPSS v12 (IBM) software.

Further analysis on the data from sublethal toxicity on longevity and residual exposure bioassays in field trials were conducted on software R (4.3.0).[50] Survival curves were generated through Kaplan–Meier model and Log-Rank pairwise comparisons were carried out using survival-package.[51,52] To test the toxicity of residual exposure, generalized linear mixed models built with the 'glmmTMB' package[53] were used. The response variable, modelled with a binomial distribution, was represented by the ratio between death and total parasitoids. The categorical explanatory variables were treatment, time, and their interaction. Random effects were included to address potential correlations within experimental plots. Models were validated by analyzing both observed and simulated residuals and conducting tests for autocorrelation. Post-hoc pairwise comparisons were conducted using the 'emmeans' package,[54] applying Holm's adjustment for multiple comparisons. Field data were plotted using 'ggplot2' package.[55]

3 RESULTS

3.1 Baseline toxicity bioassays

The probit models were fitted to the observed data for all the treatments. No significant differences between observed and expected data were found ($P > 0.05$), thus the estimations of LC_{50}, LC_{30}, and LC_5 were considered valid (Table 2). Comparing LC_{50} values, cyantraniliprole resulted the least toxic molecule, showing the highest concentration required to experimentally kill 50% of the treated wasps. On the contrary, the highest toxicity was recorded for spinosad followed by λ-cyhalothrin, phosmet and acetamiprid (Fig. 1).

The ratio between lethal concentration and maximum field rate (FR) (LC/FR) shown in Table 2, confirmed the highest toxicity of spinosad, with a LC/FR ratio of 3.72×10^{-3} for LC_{50}. Cyantraniliprole was the least toxic molecule at field rate, with a LC/FR ratio of 10.53 for LC_{50}. LC/FR values at LC_{50} of the other products ranged from 4.94×10^{-3} for phosmet, 2.56×10^{-2} for λ-cyhalothrin and 7.65×10^{-2} for acetamiprid.

3.2 Sublethal toxicity on longevity

In the untreated control, wasps mean longevity ranged from 48 ± 1.7 to 83 ± 2.2 days for male (Fig. 2) and female (Fig. 3), respectively. Male insects treated with LC_5 of the different insecticides did not show sublethal effects on longevity except for cyantraniliprole, where longevity was reduced by 65%, to a mean lifespan of 17 ± 3.8 days ($P < 0.01$) (Fig. 2). Female adults' longevity was not negatively affected by the application of LC_5 of any product ($P > 0.05$). LC_{30} of cyantraniliprole, spinosad, and λ-cyhalothrin significantly reduced male longevity to 8 ± 1 ($P < 0.01$), 6 ± 3.3 ($P < 0.01$), and 33 ± 2.7 ($P < 0.01$) days, respectively (Fig. 2). This reduction corresponded to a life decrease of 84%, 87%, and 30%. LC_{30} of cyantraniliprole and λ-cyhalothrin also significantly reduced female longevity to 45 ± 7.5 ($P < 0.01$) and 46 ± 7.5 ($P < 0.01$) days, respectively (Fig. 3). This reduction corresponded to a life decrease of 45% and 44%.

3.3 Toxicity of residual exposure in field trials

During the entire field trial with grapevine, the control group showed a mortality from 2.5 ± 5% (SD) to 10 ± 0% throughout the different assessments (Fig. 4). At T_0 and T_3, all treatments showed significant higher mortality compared to the control. At T_0 spinosad and deltamethrin were the most toxic active ingredients, causing 92.5 ± 5% and 92.5 ± 9.6% mortality, respectively, while cyantraniliprole resulted in 52.5 ± 15% mortality. Three days after treatment, residues of spinosad exhibited higher mortality compared to cyantraniliprole (87.5 ± 9.6% *versus* 37.5 ± 9.6%). One week after treatment, the residual effect of cyantraniliprole was not significantly different from the control, while spinosad and deltamethrin residues caused higher mortality of 40 ± 12% and 57.5 ± 9.6%, respectively. Fourteen days after treatment, only deltamethrin residues showed higher mortality compared with control, resulting in 57.5 ± 9.6% mortality. Twenty-one days after treatment, none of the insecticides' residues had a significant effect on *G. brasiliensis* mortality.

During the field trial with cherry, the control group showed a mortality from 10 ± 8.2% to 20 ± 8.2% across the different assessments (Fig. 5). At T_0, spinosad and deltamethrin residues showed the highest toxicity (92.5 ± 9.6%), while cyantraniliprole resulted in 47.5 ± 17.1% parasitoid mortality. Three days after treatment, the residual effects of all insecticides—spinosad, cyantraniliprole and deltamethrin—were statistically comparable, resulting in

Table 2. Baseline toxicity of the five insecticides tested resulting from the probit analysis

Active ingredient	Field rate (g a.i./L)*	Slope ± SE†	χ^2 (df)	N‡	P	LC (95% FL) (g a.i./L)§	LC/FR¶
Acetamiprid	0.11	4.290 ± 0.490	3.953 (2)	240	0.139	$LC_{50} = 8.42 \times 10^{-3}$ ($6.71 \times 10^{-3} - 1.06 \times 10^{-2}$)	7.65×10^{-2}
						$LC_{30} = 4.7 \times 10^{-3}$ ($3.51 \times 10^{-3} - 5.93 \times 10^{-3}$)	4.27×10^{-2}
						$LC_5 = 1.35 \times 10^{-3}$ ($7.65 \times 10^{-4} - 1.98 \times 10^{-3}$)	1.22×10^{-2}
Cyantraniliprole	0.075	0.224 ± 0,125	2.724 (2)	240	0.255	LC_{50} = 0.79 (0.651–1.03)	10.53
						LC_{30} = 0.45 (0.37–0.55)	6.07
						LC_5 = 0.14 (0.08–0.20)	1.87
Phosmet	0.75	14.184 ± 1.735	5.897 (2)	298	0.052	$LC_{50} = 3.70 \times 10^{-3}$ ($3.45 \times 10^{-3} - 4.01 \times 10^{-3}$)	4.94×10^{-3}
						$LC_{30} = 3.01 \times 10^{-3}$ ($2.79 \times 10^{-3} - 0.003.21 \times 10^{-3}$)	4.02×10^{-3}
						$LC_5 = 1.94 \times 10^{-3}$ ($1.63 \times 10^{-3} - 2.17 \times 10^{-3}$)	2.58×10^{-3}
λ-cyhalothrin	0.02	5.583 ± 0.657	0.083 (2)	240	0.959	$LC_{50} = 5.13 \times 10^{-4}$ ($4.45 \times 10^{-4} - 5.92 \times 10^{-4}$)	2.56×10^{-2}
						$LC_{30} = 3.12 \times 10^{-4}$ ($2.54 \times 10^{-4} - 3.65 \times 10^{-4}$)	1.56×10^{-2}
						$LC_5 = 1.08 \times 10^{-4}$ ($6.74 \times 10^{-5} - 1.47 \times 10^{-4}$)	5.40×10^{-3}
Spinosad	0.12	10.61 ± 1.164	1.107 (2)	298	0.575	$LC_{50} = 4.46 \times 10^{-4}$($3.74 \times 10^{-4} - 5.17 \times 10^{-4}$)	3.72×10^{-3}
						$LC_{30} = 3.04 \times 10^{-4}$ ($2.38 \times 10^{-4} - 3.64 \times 10^{-4}$)	2.53×10^{-3}
						$LC_5 = 1.35 \times 10^{-4}$ ($8.60 \times 10^{-5} - 1.81 \times 10^{-4}$)	1.13×10^{-3}

*Maximum Field Rate according to insecticide label expressed in g a.i./L.
† Slope and standard error of the concentration–mortality regression line.
‡ Number of tested insects excluding control.
§ Lethal concentrations and the 95% fiducial limits.
¶ Ratio between Lethal Concentration (LC) and Maximum Field Rate (FR).

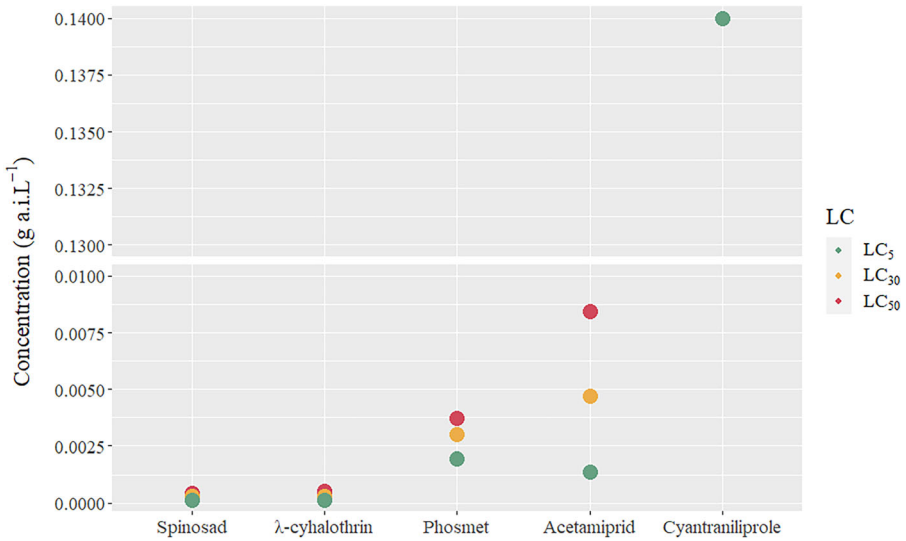

Figure 1. Visual representation of lethal concentrations (g a.i./L) of the five different products tested, ordered from the most toxic (left) to the least toxic (right) according to resulting LC_{50}. Cyantraniliprole LC_{50} and LC_{30} have been omitted to improve readability.

40 ± 8.2%, 42.5 ± 9.6% and 62.5 ± 5% *G. brasiliensis* mortality. One week after treatment, cyantraniliprole was the only a.i. not significantly impacting parasitoid survival, whereas spinosad and deltamethrin resulted in higher mortality rates of 40 ± 14.1% and 47.5 ± 5%, respectively. At 14 days after application only deltamethrin residues had a significant negative effect on parasitoids, causing a 52.5 ± 20.6% of mortality.

4 DISCUSSION

Ganaspis brasiliensis has been first released as BCA in Europe in 2021,[15,17] but currently there are no studies assessing its susceptibility toward pesticides exploited to suppress its host, *D. suzukii*. The insecticide screening performed in this study allowed us to identify the most appropriate products to be used in a CBC program perspective, reducing adverse effects on the beneficial non-target *G. brasiliensis*.

The insecticide evaluation revealed a high toxicity towards spinosad in both topical and residual exposures. This is in accordance with previous studies that classified the Hymenopteran as the most susceptible order to this molecule.[32] The aphid parasitoid *Aphidius colemani* (Viereck) (Hymenoptera: Braconidae) for instance was nearly 20 times more susceptible to the bio-insecticide spinosad than the conventional insecticides, imidacloprid and λ-cyhalothrin.[56] The pupal parasitoid *P. vindemiae*, was found to be highly susceptible to spinosad when it came into direct contact with low concentration of the product (10 mg a.i/L).[33] Topical bioassays conducted with four insecticides revealed that

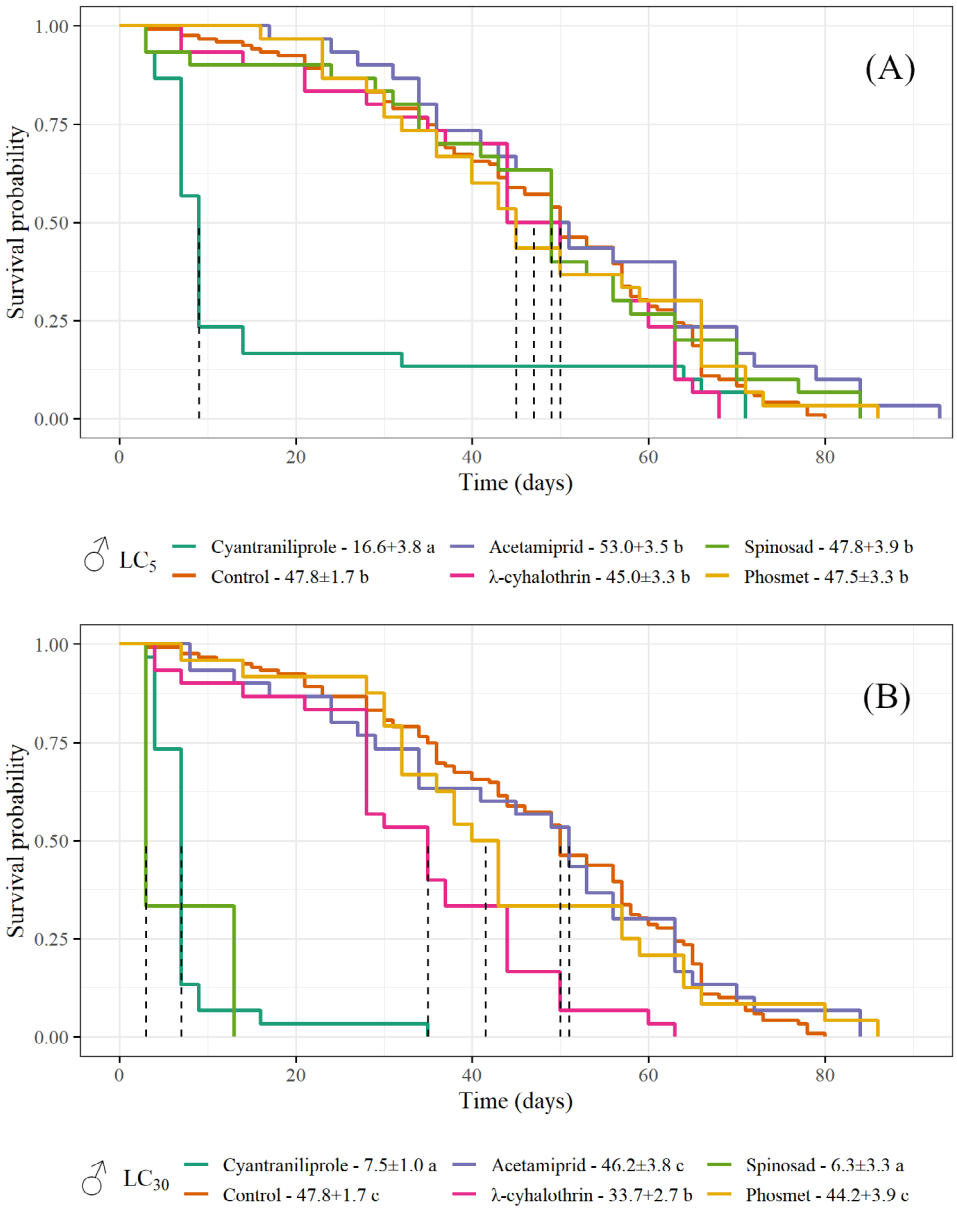

Figure 2. Survival curve of male adult *Ganaspis brasiliensis* exposed to LC_5 (A) and LC_{30} (B) of the five tested insecticides. The curves were generated through Kaplan–Meier estimators and compared in the Log-Rank test ($P < 0.05$). Different letters identify significant different curves. Numbers close to the product names are the mean average mortality expressed in days ± SEM. Vertical dotted lines show the median mortality.

spinetoram was the most harmful to a closer relative of our target species, the figitid wasp *Ganaspidium nigrimanus* (Kieffer) (Hymenoptera: Figitidae), larval-pupal parasitoid of the leaf miner fly *Liriomyza trifolii* (Burgess) (Diptera: Agromyzidae).[57] Our topical experiments revealed higher susceptibility of male wasps towards sublethal concentrations (LC_{30}) of spinosad. Moreover, both field trials showed a significant higher mortality of male wasps compared to females (Data not shown). Higher male susceptibility to insecticide is known in *D. suzukii*,[48,58] and previously reported for *G. nigrimanus*.[57] The cause of this difference has been explored in other studies, where it has been attributed to sexual dimorphism[59,60] or activity of detoxification enzymes.[61]

In our residual assay conducted in the field, spinosad showed residual activity up to 7 days after application in both grapevine and cherries. This time range is consistent with previous literature, although spynosyn's persistence varies greatly across different matrixes, with half-life ranging from 1.2 days on Chilli to over 16 days on Kiwi fruit.[62] Sunlight strongly contributes to spinosad dissipation, potentially explaining the gradual decrease in mortality recorded in the field.[63]

Spinosad has been proven effective to suppress *D. suzukii*, with LC_{90} at 48 h at 60.08% (7.21×10^{-2} g a.i./L) of the recommended field rate.[64] Our results suggest that, even at this lower concentration, spinosad would be extremely toxic to *G. brasiliensis* adults. Our findings show that spinosad has a high knockdown effect in both topical and residual assays, but also affects longevity when sprayed at lower concentration, specifically in males. This underscores the incompatibility of this active ingredient to biological control programs involving the release of *G. brasiliensis* as a BCA.

The pyrethroids, λ-cyhalothrin and deltamethrin, were also highly toxic to *G. brasiliensis*. λ-cyhalothrin and spinosad showed similar lethal concentrations, indicating equal toxicity of their

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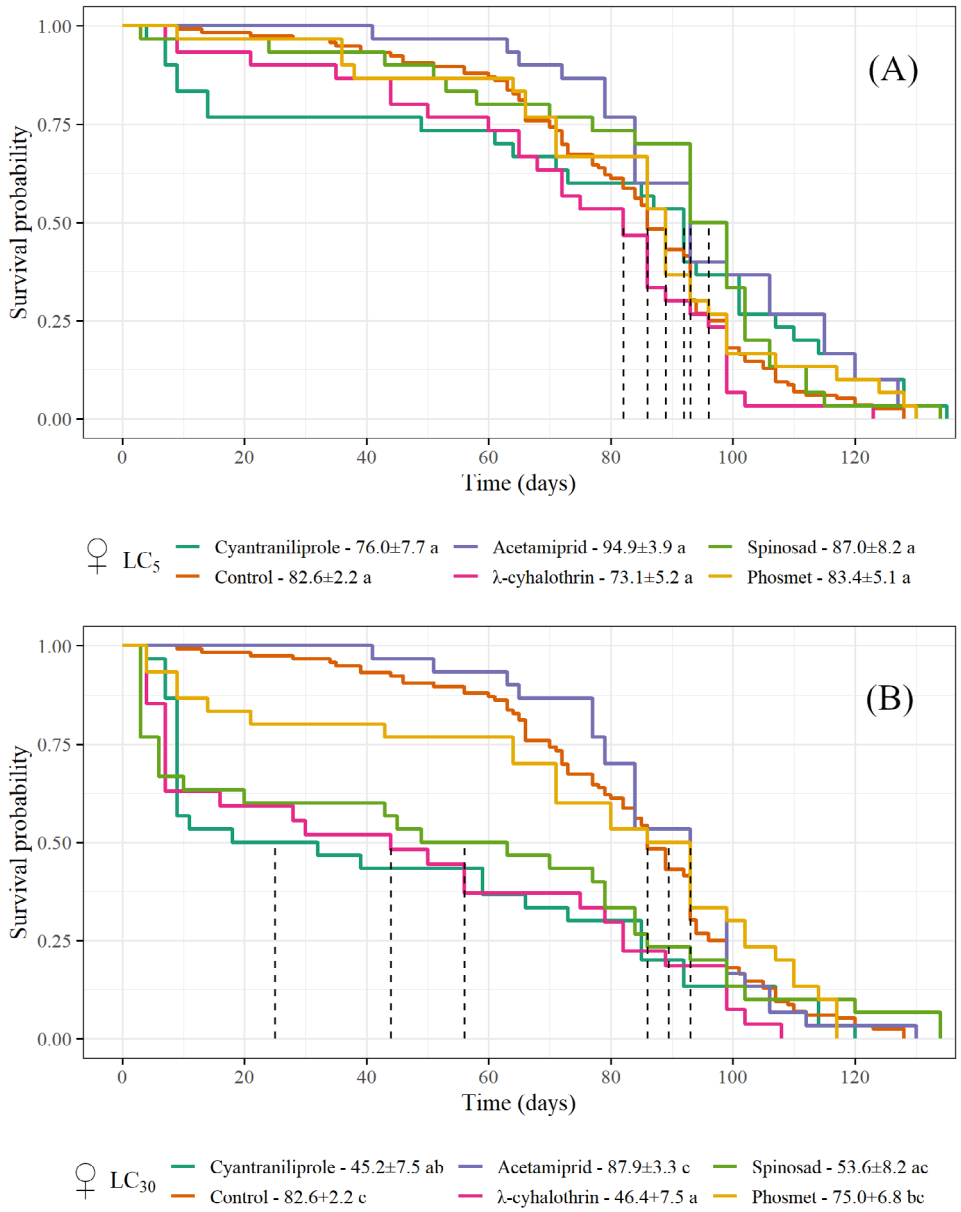

Figure 3. Survival curve of female adult *Ganaspis brasiliensis* exposed to LC_5 (A) and LC_{30} (B) of the five tested insecticides. The curves were generated through Kaplan–Meier estimators and compared in the Log-Rank test ($P < 0.05$). Different letters identify significant different curves. Numbers close to the product names are the mean average mortality expressed in days ± SEM. Vertical dotted lines show the median mortality.

active ingredients. However, because of the recommended field rate, the LC/FR ratio for λ-cyhalothrin was higher than for spinosad, making its field use theoretically less toxic. The high knockdown effect caused by λ-cyhalothrin in the topical assays is likely due to the mode of action of this molecule, which quickly permeates the epidermis to reach insect nervous system.[65,66] In the field trials, deltamethrin had the most persistent effect on insect mortality, showing toxicity up to 14 days after insecticide application. This is consistent with previous studies showing persistence of deltamethrin residues on tea leaves up to 14 days.[67] The reduction in toxicity observed in our study from day 3 onward is in accordance with the product half-life of 3.04 days,[67] but the high mortality rates 7 and 14 after treatment suggests that residues can also be highly toxic. The residual impact of this insecticide group is also evident in the reduced longevity observed in both male and female wasps treated with LC_{30} of λ-cyhalothrin.

Similar to spinosad, pyrethroids demonstrated high toxicity in both topical and residual assays, suggesting that their field application could be detrimental to CBC programs. Compared to spinosad, λ-cyhalothrin is more toxic towards *D. suzukii*, with LC_{90} at 48 h estimated at 24.66% (4.93×10^{-3} g a.i./L) of the field rate.[64] According to our results, even at this lower concentration, *G. brasiliensis* would be strongly affected.

Cyantraniliprole was the least toxic active ingredient, in both topical and residual assays. This outcome is supported by the fact that often broad-spectrum insecticides affecting the nervous and respiratory systems are more toxic towards parasitoids compared to selective ones compromising insect growth.[23] Cyantraniliprole is a second-generation ryanodine receptor modulator that possess the capacity to bind and stimulate receptors within the insects' muscle cells, causing contraction, paralysis, and ultimately death.[68,69] This mode of action leads to a slower death, as it

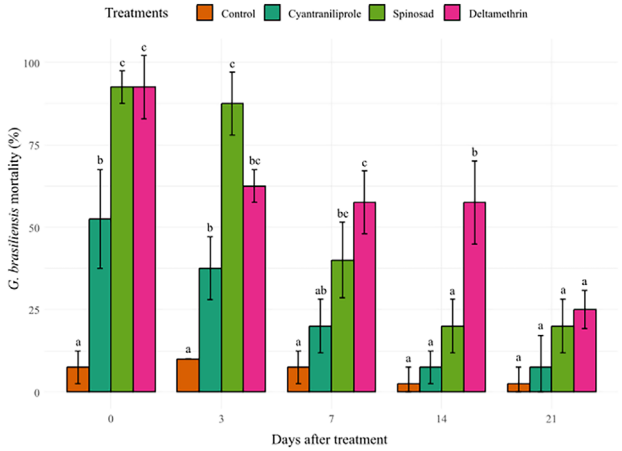

Figure 4. Residual effects of tested active ingredients on *Ganaspis brasiliensis* observed in the grapevine field trial 0, 3, 7, 14 and 21 days after insecticides application. Mortality (%) is reported as mean ± standard deviation represented by error bars. Treatments labelled with different letters are statistically different at $P < 0.05$.

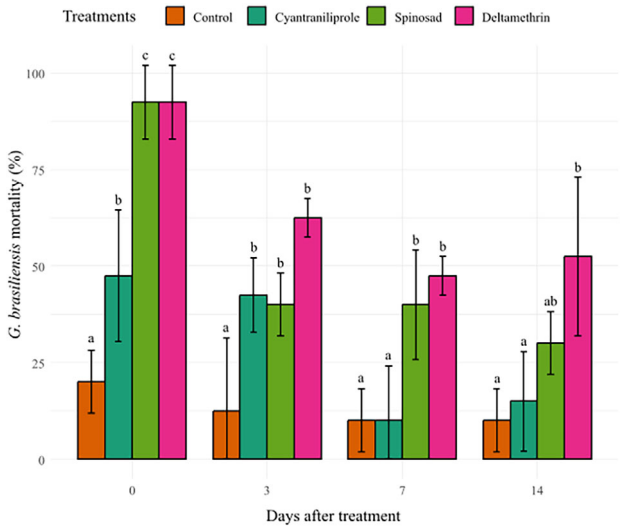

Figure 5. Residual effects of tested active ingredient on *Ganaspis brasiliensis* observed in the cherry field trial 0, 3, 7 and 14 days after insecticides application. Mortality (%) is reported as mean ± standard deviation represented by error bars. Treatments labelled with different letters are statistically different at $P < 0.05$.

gradually impedes the insect's ability to move and feed. For instance, residual exposure of cyantraniliprole at field rate was found scarcely toxic to the adults of *Cydia pomonella* L. (Lepidoptera: Tortricidae), but negatively affected insect movement and mating.[70] Similarly, it disrupts feeding in white fly adults, *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae).[71] In the baseline bioassay, cyantraniliprole showed low toxicity but caused high reduction of longevity in the sublethal assessments. This can be attributed to the slow knock-down effect of cyantraniliprole on the tested parasitoids and the 48-h mortality assessment period selected for the bioassays. The high mortality recorded at LC_{30} starting a week after application suggests that mortality assessment for this active ingredient should be conducted later, as done in previous studies on stored product pests, where mortality was checked at 7 and 28 days after pesticide application.[72] A later assessment would have probably revealed higher baseline toxicity and lower LC values, resulting in increased adult longevity in the sublethal effect trial.

Cyantraniliprole formulation tested in the field trials has been proven effective in suppressing *D. suzukii* with a 90% mortality at 20.56% (equivalent to: 0.015 g a.i./L) of the field rate.[64] Considering our findings and the mortality recorded in the bioassays and field trials, cyantraniliprole can be considered the most selective insecticide tested. In fact, field rates that would cause >90% mortality in *D. suzukii* only resulted in 52.5 and 47.5% *G. brasiliensis* mortality in vineyard and cherry orchard, respectively.

Phosmet active ingredient was the third most toxic. But considering the high concentration applied at maximum field rate, it would have been the second most toxic product based on LC/FR ratio. Given the documented health hazard posed by phosmet to human and other organisms,[73] and its withdrawal from use in Europe,[46] the product has not been further evaluated.

One of the major concerns undermining control management efforts is the development of insecticide resistance.[58,74] Studies have shown that some *D. suzukii* populations in America have developed resistance to spinosad[28,75,76] and pyrethroids.[77] Selection bioassays conducted on Italian populations revealed that after eight generations, *D. suzukii* was less susceptible to deltamethrin and cyantraniliprole (LC_{50} values increased 25.0 and 2.2-fold, respectively).[78] For all the active ingredients tested in our field trials, resistance has been recorded, indicating a future need to increase insecticide concentrations to maintain effectiveness and the necessity to utilize multiple insecticides with different mode of actions.[74,79] On the other hand, very few cases of pesticide resistance have been documented for parasitoids compared to pests.[79] This is likely due to the fewer generations per year they produce. In fact, in our temperate climate we estimate the occurrence of five generations of *G. brasiliensis* compared with the seven to 15 estimated for *D. suzukii*.[2] This might result in slower accumulation of genetic diversity within the population, including potential resistance-conferring mutations. If this is the case, the resistance gap to insecticides between *G. brasiliensis* and *D. suzukii* will likely increase. However, more studies are required to test this hypothesis, as the influence of generation time on resistance evolution cannot be generalized.[80]

The timing of pesticide application is crucial for effectively targeting pests while minimizing adverse effects on non-target species. This is challenging, as the close evolutionary bond between parasitoids and their hosts often results in their overlapping presence in the field.[35] *Drosophila suzukii* can develop at lower temperatures, with a thermal threshold of 8.1 °C,[81] while *G. brasiliensis* (G3 strain from South Korea) enters diapause at temperatures below 17.2 °C.[82] Although there is limited literature on the overwintering performance and diapause exit of *G. brasiliensis*, we can infer that in spring, there will be a period when *D. suzukii* is actively developing and colonizing new habitats while *G. brasiliensis* remains dormant. In temperate climates *D. suzukii* overwintering females with mature eggs can be collected as early as late February.[83] They move from forest habitat to cultivated orchards after the cold season (April–June),[84] and *G. brasiliensis* might take longer to follow its dispersal due to dormancy and the longer development time (476.2 *versus* 222.2° days).[82] Early season application of adulticides is estimated to be more effective in reducing *D. suzukii* population growth[85] and might be the best option to minimize adverse effects on *G. brasiliensis*. However, it is important to note that most commercial crops fruit in summer when both species are likely to coexist in the same agroecosystem. Therefore, choosing the most selective pesticides is essential.

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In this scenario, habitat management could be an effective strategy to prevent the depletion of *G. brasiliensis* population following pesticide application. Thanks to its ability to develop into numerous wild species,[86] *D. suzukii* heavily relies on wild habitat, such as surrounding vegetation[87] and forests.[84] Although marginal landscape complexity can be seen as counter-productive,[88] it also provides refuge for beneficials. A feasible option to support the *G. brasiliensis* population while avoiding an increase in pest damage is the adoption of augmentoria[89] in untreated portions of the field or along natural hedgerows. This technique allows for maintaining a safe reservoir of host juveniles to support parasitoid population year-round, and has already been proven effective to increase control pressure on *D. suzukii*.[90]

Both field trials yielded consistent results, although in the cherry trial, mortality was assessed only up to 14 days after the treatment due to a shortage of cherries on the plants. Overall, higher temperatures were recorded during the cherry trials, although fluctuations occurred throughout the experimental period. The average mean temperature in cherry trial was 22.7 ± 2.7 °C, whereas in the grapevine trial it was 18.1 ± 2.9 °C (Fig. S1 in Supporting Information).

This study offers valuable guidance for integrating BCA within an IPM framework by presenting the results of topical and residual toxicity bioassays and field trials. While we assessed sublethal effects on adult longevity, further research should evaluate other physiological and behavioral responses to fully understand the toxicity of active ingredients toward *G. brasiliensis*. These might affect also immunology, fecundity, sex ratio, mobility, feeding, and oviposition.[91] We performed an additional trial to test whether residual insecticide application affected *G. brasiliensis* offspring production in sweet cherry orchard. Results (available in the Supporting Information) show a reduction of offspring for all tested products compared to control (Fig. S2), in accordance with the bioassays. These preliminary results can be a starting point to further investigate if the reduction we observed is due to higher adult mortality, or if chemical application altered parasitization success or insect fecundity, as previously reported for *D. suzukii* pupal parasitoids.[30,31]

5 CONCLUSIONS

To the authors knowledge this is the first ecotoxicology screening performed on the non-target *G. brasiliensis*, and can provide a basis for insecticide selection in an area subjected to a classical biological control program. According to our results, spinosad is the most toxic among the product tested, with a high knock-out effect in both laboratory and field trials. λ-cyhalothrin was also highly toxic and its application at LC_{30} significantly reduced both male and female longevity. In field trial, deltamethrin showed the most prolonged residual effect, causing higher mortality up to 14 days after treatment. Cyantraniliprole was the least toxic active ingredient in both topical and residual bioassays, suggesting its potential as the most selective option among the tested insecticides. Given that the parasitoid complex of this pest is rapidly moving towards invaded territories,[16,92,93] it is important for future studies to evaluate insecticide susceptibility of other major *D. suzukii* parasitoids, such as *Leptopilina japonica* (Novković and Kimura) (Hymenoptera: Figitidae).

ACKNOWLEDGEMENTS

We thank M. L. Seehausen and M. Kenis from CABI, Delémont, Switzerland, for providing the author MVRS, Fondazione Edmund Mach, San Michele all'Adige, Italy, the initial colony of the Japanese strain of *Ganaspis brasiliensis* G1. The study was supported by the SWAT Project (Fondazione Edmund Mach with the contribution of the Autonomous Province of Trento), and the Veneto Region with the contribution of FMC Agro Italia. LF PhD fellowship is funded by Fondazione Edmund Mach, the University of Trento, Laimburg Research Center and Sant'Orsola s.c.a. GDZ PhD scholarship is funded by REACT-EU FSE in the frame of PON 'Ricerca e Innovazione' 2014–2020 (DM 1061/2021). Codice BIO12, DOT1340225, Borsa 1 CUP B39J21026610001. Open access publishing facilitated by Fondazione Edmund Mach Istituto Agrario di San Michele all'Adige, as part of the Wiley - CRUI-CARE agreement.

CONFLICT OF INTEREST

The authors declare no competing financial interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

SUPPORTING INFORMATION

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

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