

# Impact of direct and indirect ingestion of six systemic pesticides on the parasitoid *Anagyrus vladimiri*

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## ABSTRACT

*Anagyrus vladimiri* (Hymenoptera: Encyrtidae) is the main parasitoid of *Planococcus citri* (Hemiptera: Pseudococcidae), which is one of the most important pests of citrus crops in the Mediterranean Basin. Pesticide residue on trees and in the nectar of wildflowers growing in citrus orchards might cause side effects in beneficial insects. In the present study, we assessed the effects of six systemic pesticides (acetamiprid, dimethoate, cyantraniliprole, flupyradifurone, spirotetramat and sulfoxaflor) on the survival and progeny of *A. vladimiri*. *Anagyrus vladimiri* fed on the pesticide solutions and on the nectar of *Fagopyrum esculentum* Moench plants that had been watered with a solution containing these pesticides. Acetamiprid, dimethoate, flupyradifurone and sulfoxaflor increased female mortality more than the control (water) after 6h; after *A. vladimiri* consumed these pesticides, female mortality ranged from 62% to 73%. Cyantraniliprole had no immediate toxic effect on parasitoid survival but caused mortality after 15 days. All tested pesticides except flupyradifurone and spirotetramat reduced parasitoid progeny. After six days of feeding on *F. esculentum* nectar of plants watered with a pesticide solution, significantly increased female mortality was observed with dimethoate (96%), spirotetramat (80%), acetamiprid (62%), cyantraniliprole (62%) and sulfoxaflor (50%). Progeny was reduced in females that fed on plants treated with spirotetramat and dimethoate. These results demonstrate that some of these insecticides can have lethal or sublethal effects on *A. vladimiri* by both routes of exposure, via direct or indirect pesticide consumption through the nectar of treated plants.

## 1. Introduction

Citrus orchards, as perennial tree crops, can host a wide range of pests throughout the year, as well as those pests' natural enemies. Most citrus pests can be successfully controlled by a guild of parasitoids and predators, which keep pests below their economic thresholds (Jacas and Urbaneja, 2010; Urbaneja et al., 2020). Conservation biological control (CBC), which aims to protect and enhance the effectiveness of natural enemies in reducing pest populations by modifying the environment or existing agricultural practices (Eilenberg et al., 2001), is a key tool in citrus orchards that use integrated pest management (IPM). Resident or sown vegetation covers are an important component of CBC, as they provide a food source and shelter for beneficial insects and help conserve pests' natural enemies (Silva et al., 2010; Gómez-Marco et al., 2016; IOBC/WPRS et al., 2022).

The citrus mealybug *Planococcus citri* (Risso) (Hemiptera: Pseudococcidae) is an occasional pest in the Mediterranean citrus growing area

due to efficient control by natural enemies, which usually maintain the pest population below the economic threshold (Urbaneja et al., 2020). One natural enemy that plays an important role in the control of *P. citri* is the parasitoid *Anagyrus vladimiri* Triapitsyn (Hymenoptera: Encyrtidae) (Jacas and Urbaneja, 2010). This successful control may provide a case study for other mealybug pests and *Anagyrus* spp. parasitoids.

Citrus agroecosystems are dynamic, and exotic species are frequently introduced. Globalization and climate change have intensified this trend (Peña, 2013). One noteworthy case is the impact of the South African mealybug, *Delottococcus aberiae* (De Lotto) (Hemiptera: Pseudococcidae) on the Spanish citrus industry. After its detection in 2009 (Beltrà et al., 2013) and dispersal over the following decade throughout many provinces on the eastern coast of Spain (Navarro-Llopis et al., 2019), it has become the species with the greatest economic impact on citrus crops (Beltrà et al., 2013). The main threat to the Mediterranean citrus industry is the citrus disease Huanglongbing (HLB). One of its vectors, the African citrus psyllid *Trioza erytreae* Del Guercio (Hemiptera: Triozidae),

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was detected in the Iberian Peninsula in 2014 (Pérez-Otero et al., 2015). Currently, *T. erytrae* is present in South Portugal (Algarve), very close to the Mediterranean citrus growing area (EPPO Global Database, 2021; DGVA, 2023). Thus, repeated use of pesticides is sometimes needed to control both endemic and exotic pest species, which can disrupt the biological control of citrus pests. Therefore, to plan a control strategy for exotic pests, it is crucial to determine which pesticides and which routes of pesticide exposure have the smallest impact on the natural enemies used in citrus IPM programs.

Natural enemies are directly exposed when pesticides are applied or through pesticide residues. The first toxic impact on natural enemies occurs via pesticide contact and/or inhalation. This may occur when the tree canopy is treated, during topical exposure to pesticides or when natural enemies walk on dried residues (Suma et al., 2009; Barros et al., 2018). Natural enemies may also be exposed by feeding on pesticide-treated prey (Martinou et al., 2014; Rugno et al., 2021) or by ingesting directly contaminated food sources such as pollen, nectar, honeydew or guttation drops (Cloyd and Bethke, 2011; Calvo-Agudo et al., 2019; Martínez-Ferrer et al., 2019). Furthermore, since 29% of pesticides that are sprayed on the tree canopy reach the ground (Garcera et al., 2017), systemic pesticide residue can also be present on ground cover plants with flowers, and this residue can eventually translocate to pollen and nectar (David et al., 2016; García-Valcárcel et al., 2022). Neonicotinoids have been detected in wildflower nectar more than 200 days after their application in citrus orchards (García-Valcárcel et al., 2022). Consumption of contaminated wildflower nectar may lead to lethal or sublethal side effects in natural enemies (Desneux et al., 2007), reducing or limiting their ability to regulate pest populations.

The toxicity of several pesticides on *A. vladimiri* and *Anagyrus* spp. has been previously assessed, primarily focusing on exposure when the pesticide is applied (Mansour et al., 2023; Mgocheki and Addison, 2015; Satar et al., 2018; Singh et al., 2023). These studies have shown how contact with the pesticide can cause mortality and reduce the progeny of *Anagyrus* spp. Furthermore, pesticides also can have a toxicity impact on *Anagyrus* spp. when feeding on contaminated food, i.e., by direct ingestion when applied (Singh et al., 2023) or when feeding on honeydew contaminated with pesticide residues (Calvo-Agudo et al., 2019). However, *Anagyrus* spp. and other natural enemies can also be exposed to additional contaminated routes that are often overlooked. This includes residues of pesticides that reach the nectar of wildflower plants in groundcover via systemic pathway resulting from drift during crop treatments (García-Valcárcel et al., 2022).

This study aimed to assess the potential side effects of the described systemic pesticides on the survival, longevity and progeny of the parasitoid *A. vladimiri*. The impacts on *A. vladimiri* of direct pesticide ingestion and of indirect toxicity via ingestion of the nectar of treated *F. esculentum* flowers were examined.

2. Materials and methods

2.1. Plants and insects

Buckwheat, *Fagopyrum esculentum* Moench, was selected for its flower morphology, which allows easy access to the nectaries (Cawoy et al., 2008). In previous experiments, we confirmed that *A. vladimiri* females were able to feed on the floral nectar of this plant (personal observations), in addition to this being shown in other studies (Krischik et al., 2007; Irvin and Hoddle, 2015). Plants were seeded directly in pots (3L) and grown to a height of 40cm–60cm.

*Planococcus citri* females were reared at the Institute of Agrifood Research and Technology (IRTA) in Amposta (Tarragona, Spain) on potato shoots in a climatic chamber at 24.4 ± 0.5 °C, with 66 ± 6.5% relative humidity (RH) and a photoperiod of 16:8 (L:D). *Anagyrus vladimiri* individuals were purchased from Koppert® (Citripap, Koppert Biological Systems, The Netherlands) and then raised on *P. citri* females at IRTA-Amposta facilities under the same climatic conditions as *P. citri*.

All parasitoid females used in the experiments were 0–72h old.

2.2. Direct ingestion of pesticides: solutions experiment

Six systemic pesticides were selected based on their efficacy against hemipteran pests, especially *T. erytrae* (Molina et al., 2022): acetamiprid, cyantranilprole, dimethoate, flupyradifurone, spirotetramat and sulfoxaflor. They were used at the maximum application rate (Table 1), and water was used as control treatment. Sugar (25%) was added to all solutions to ensure parasitoid consumption. Each treatment solution was placed in a plastic vial (50mL) with a permeable wick in the lid to allow parasitoids to feed, and those plastic vials were placed in a bigger one (Ø = 11cm, h = 10.5cm) with a ventilation lid. Ten *A. vladimiri* females that had not fed for 24h were used per replicate, and four replicates of each treatment were conducted. The number of living and dead females after 3h and 6h were recorded to assess mortality.

Next, the surviving *A. vladimiri* females were individually placed in ventilated plastic vials (Ø = 3cm, h = 7 cm). They were only fed with sugar–water solution (25%) as a food source, offered on a cotton swab that was replaced twice a week. The number of living and dead females was recorded daily for 15 days. Up to 20 replicates were conducted in those treatments where more than 20 females survived.

Furthermore, other *A. vladimiri* females were allowed to feed on pesticide solutions for 6h. After that, each female was placed in a ventilated plastic vial (Ø = 6cm, h = 3cm) with two *A. vladimiri* males and offered at least 30 young females of *P. citri* on a piece of potato shoot with some honey drops as a food source. Four days later, the parasitoids were removed from the vials, and the numbers of living and dead individuals were recorded. Females of *P. citri* were kept until parasitoid emergence, and progeny and gender were then recorded. Sixteen to 20 replicates were conducted for each treatment. All experiments were conducted under controlled conditions (24.41 ± 0.5 °C; 66 ± 6.5% RH; 16:8 [L:D] photoperiod).

2.3. Indirect ingestion of pesticides: plants experiment

This experiment was conducted in an outdoor facility at IRTA-Amposta, with proper ventilation and protected from direct solar radiation. Pesticides were applied by watering fully bloomed buckwheat plants at the maximum permitted rate per hectare (Table 1). Each plant was watered with 50mL of the pesticide solution, water was used as a control. The buckwheat plants were placed on shelves and covered with a mesh to prevent insects from feeding on the flowers. They were then irrigated daily, avoiding percolation, for 3 days. The plant substrate was covered with expanded polystyrene to hinder parasitoid access. Next, each plant was placed in a cage (60 x 60 x 60cm) with ten *A. vladimiri* females. After 6 days, the numbers of living and dead *A. vladimiri* females were recorded. Three sets of trials (August, September and October) were carried out, each with four replicates per treatment.

Table 1  
Pesticide solutions used in the direct ingestion experiment.

| Active Ingredient | Trade Name   | Manufacturer | Formulation <sup>a</sup> | Rate    |
|-------------------|--------------|--------------|--------------------------|---------|
| Acetamiprid       | Carnadine®   | Nufarm       | 20% SL                   | 0.25g/L |
| Cyantranilprole   | Exirel®      | FMC          | 10% SE                   | 1mL/L   |
| Dimethoate        | Perfekthion® | BASF         | 40% EC                   | 1mL/L   |
| Flupyradifurone   | Sivanto®     | Bayer        | 20% SL                   | 0.5mL/L |
| Spirotetramat     | Movento® 150 | Bayer        | 15% OD                   | 0.4mL/L |
| Sulfoxaflor       | Closer®      | CropScience  |                          | L       |
|                   |              | Corteva      | 11.3% SC                 | 0.4mL/L |
|                   |              | Agrisience   |                          | L       |

<sup>a</sup> SL: soluble concentrate; SE: suspoemulsion; EC: emulsifiable concentrate; OD: oil dispersion; SC: suspension concentrate.

Average temperatures and RH% were  $26.2 \pm 0.2$  °C and  $80.3 \pm 0.7\%$  in August,  $21.3 \pm 0.2$  °C and  $70.8 \pm 1.0$  % in September, and  $17.9 \pm 0.2$  °C and  $78.7 \pm 0.6$  % in October. Surviving females of each treatment were allowed to parasitize young female *P. citri* as explained above, and the number of progeny after 21 days was recorded.

2.4. Statistical analyses

Mortality resulting from the different pesticide solutions at 3h and 6h was analyzed using Generalized Linear Models (GLM) with a binomial distribution followed by Tukey’s HSD. For pesticides that caused significantly different survivorship rates than the control treatment, Abbott’s formula ( $Corrected\ \% = \left(1 - \frac{N\ in\ Treatment\ after\ treatment}{N\ in\ Control\ after\ treatment}\right) \times 100$ ) (Abbott, 1925) was used to determine the corrected mortality percentage. A *t*-test was used to compare mortality at 3h and 6h. A Kaplan-Meier analysis was used to estimate the survival curves of the *A. vladimiri* females, which were compared using the log-rank test and a pairwise analysis. Finally, progeny for each treatment was analysed using GLMs (Poisson distribution) based on total number of adults that emerged. Replicates in which females died before the experiment was complete were not included in the analysis. The sex ratio (emerged females/total emerged adults) was calculated for each replicate, and treatments were compared using GLMs (Gaussian distribution).

In the plants experiment, female survival was analysed using GLMs (binomial distribution); the number of alive individuals per cage was used as the variable response, and the pesticide treatment and trial month were used as explanatory variables. Next, Tukey post-hoc tests were used to perform multiple comparisons among pesticide treatments for each trial. When significant, mortality was corrected using Abbott’s formula. Progeny was measured as described above.

The GLMs were fitted using the most appropriate distribution for each dataset. All statistical analysis were conducted using R (version 4.2.0) (R Core Team, 2022); significance was set to 5%.

3. Results

3.1. Direct ingestion of pesticides: solutions experiment

The number of *A. vladimiri* females that survived after feeding directly on the pesticide solutions varied significantly among treatments at 3h ( $\chi^2 = 46.6$ , df = 6,  $p < 0.001$ ) and 6h ( $\chi^2 = 120$ , df = 6,  $p < 0.001$ ) (Table 2). After 3h, *A. vladimiri* survival in the dimethoate,

flupyradifurone and sulfoxaflor treatments were significantly lower than in the control treatment, with corrected mortality of 35.1%, 43.2% and 32.4%, respectively. Corrected mortality after 6h was 73.0% for flupyradifurone and 70.3% for dimethoate and sulfoxaflor. After 6h, females which fed on acetamiprid also had significantly lower survival rates (62.2% corrected mortality) than females in the control. Of the four pesticides that caused significant mortality in *A. vladimiri*, dimethoate and flupyradifurone caused significantly higher mortality after 6h than after 3h ( $t = 2.8$ , df = 6,  $p = 0.0333$ ;  $t = 2.8$ , df = 6,  $p = 0.0320$ , respectively). For the other two pesticides, sulfoxaflor and acetamiprid, these differences in parasitoid survival were not significant between 3 and 6h ( $t = 1.9$ , df = 6,  $p = 0.1020$ ;  $t = 1.8$ , df = 6,  $p = 0.1250$ , respectively). Fifteen days after feeding on the pesticide solutions, only *A. vladimiri* females fed on cyantranilprole survived at significantly different rates than those in the control treatment ( $\chi^2 = 22.9$ , df = 6,  $p < 0.001$ ; Fig. 1), since more than 50% of parasitoids that died did so during the first two days.

Furthermore, the progenies of females that fed 6h on pesticide solutions were negatively affected in the acetamiprid, cyantranilprole, dimethoate and sulfoxaflor treatments ( $\chi^2 = 65.7$ , df = 6,  $p < 0.001$ ), since they produced significantly less progeny than those in the control treatment (Table 2). The sex ratios of the progeny did not differ significantly among any of the treatments ( $\chi^2 = 7.73$ , df = 6,  $p = 0.26$ ).

3.2. Indirect ingestion of pesticides: plants experiment

For the number of surviving *A. vladimiri* females fed on the nectar of treated buckwheat plants, an interaction between treatments and trial sets over three months was observed ( $\chi^2 = 47.6$ , df = 12,  $p < 0.001$ ). Therefore, the three trials were analysed separately (Table 3). In the trials conducted in August, *A. vladimiri* females fed on plants watered with three of the six tested pesticide solutions (dimethoate, spirotetramat and acetamiprid) had significantly lower survival rates than those in the control treatment ( $\chi^2 = 54$ , df = 6,  $p < 0.001$ ); these three pesticides caused 96.3%, 81.5% and 63.0% of mortality, respectively. In the trials conducted in September, the treatments that differed significantly to the control were acetamiprid, cyantranilprole, spirotetramat and sulfoxaflor ( $\chi^2 = 39.4$ , df = 6,  $p < 0.001$ ), which caused 62.1%, 62.1%, 82.8% and 51.7% of mortality, respectively. Finally, in the October trials, no treatment differed significantly to the control ( $\chi^2 = 16.7$ , df = 6,  $p = 0.01$ ).

The number of adult parasitoids that emerged from females fed on

**Table 2**  
Number of living *Anagyrus vladimiri* (mean  $\pm$  SE) females 3h and 6h after the parasitoids fed on each pesticide solution (N = 10 per replicate), number of *A. vladimiri* (mean  $\pm$  SE) that emerged, and sex ratios (mean  $\pm$  SE) of the progeny for each treatment.

| Treatment       | Number of living <i>A. vladimiri</i> |                 | Emerged adults   | Sex Ratio       |
|-----------------|--------------------------------------|-----------------|------------------|-----------------|
|                 | 3h                                   | 6h              |                  |                 |
| Control         | 9.25 $\pm$ 0.75 a                    | 9.25 $\pm$ 0.75 | 31.75 $\pm$ 3.06 | 0.34 $\pm$ 0.04 |
| Acetamiprid     | 6.50 $\pm$ 1.19                      | 3.50 $\pm$ 1.19 | 21.92 $\pm$ 3.62 | 0.33 $\pm$ 0.06 |
|                 | abc                                  | b               | b                | a               |
| Cyantranilprole | 8.75 $\pm$ 0.63                      | 7.50 $\pm$ 0.65 | 21.50 $\pm$ 2.39 | 0.34 $\pm$ 0.05 |
|                 | ab                                   | a               | b                | a               |
| Dimethoate      | 6.00 $\pm$ 0.82                      | 2.75 $\pm$ 0.85 | 22.92 $\pm$ 3.77 | 0.38 $\pm$ 0.06 |
|                 | bc                                   | b               | b                | a               |
| Flupyradifurone | 5.25 $\pm$ 0.48 c                    | 2.50 $\pm$ 0.87 | 28.50 $\pm$ 2.53 | 0.49 $\pm$ 0.06 |
|                 |                                      | b               | a                | a               |
| Spirotetramat   | 9.75 $\pm$ 0.25 a                    | 9.25 $\pm$ 0.25 | 31.40 $\pm$ 4.61 | 0.39 $\pm$ 0.07 |
|                 |                                      | a               | a                | a               |
| Sulfoxaflor     | 6.25 $\pm$ 1.31                      | 2.75 $\pm$ 1.25 | 22.00 $\pm$ 4.27 | 0.38 $\pm$ 0.07 |
|                 | bc                                   | b               | b                | a               |

Different letters in each column indicate significant differences among treatments, compared using the Tukey test (5% significance level).

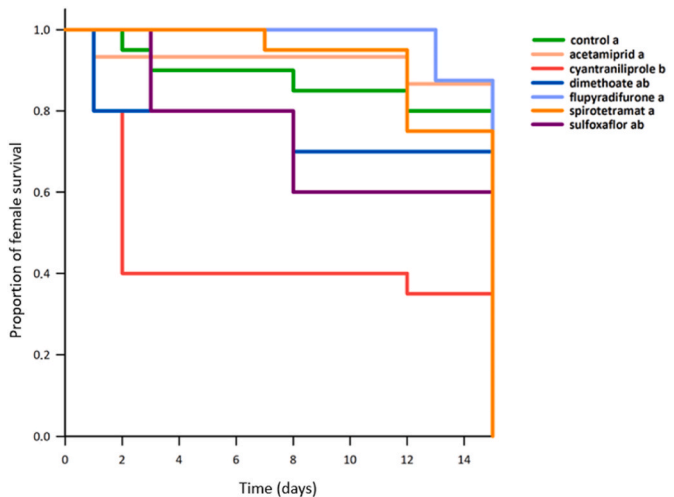


**Fig. 1.** Kaplan-Meier estimated survivorship during 15 days of *A. vladimiri* females that fed on different pesticide solutions for 6h. Different letters in the legends indicate significant differences among treatments (5% significance level).

**Table 3**

Number of living *A. vladimiri* females (mean  $\pm$  SE) per treatment and month after the parasitoids fed on the nectar of treated buckwheat plants (N = 10 per replicate). In addition, number of *A. vladimiri* (mean  $\pm$  SE) that emerged and sex ratios (mean  $\pm$  SE) of the progeny per treatment.

| Treatment        | Number of living <i>A. vladimiri</i> females |                     |                    | Emerged adults       | Sex Ratio         |
|------------------|----------------------------------------------|---------------------|--------------------|----------------------|-------------------|
|                  | August                                       | September           | October            |                      |                   |
| Control          | 6.75 $\pm$ 1.31 a                            | 7.25 $\pm$ 1.55 a   | 7 $\pm$ 0.41 ab    | 23.68 $\pm$ 1.69 ab  | 0.55 $\pm$ 0.04 a |
| Acetamiprid      | 2.5 $\pm$ 2.18 bc                            | 2.75 $\pm$ 1.8 bc   | 6.5 $\pm$ 0.87 ab  | 22.87 $\pm$ 2.38 abc | 0.52 $\pm$ 0.05 a |
| Cyantraniliprole | 3.5 $\pm$ 1.85 abc                           | 2.75 $\pm$ 2.14 bc  | 8.25 $\pm$ 0.48 a  | 26.11 $\pm$ 2.06 a   | 0.52 $\pm$ 0.04 a |
| Dimethoate       | 0.25 $\pm$ 0.25 c                            | 4.25 $\pm$ 1.55 abc | 5.75 $\pm$ 0.48 ab | 14.36 $\pm$ 2.82 d   | 0.55 $\pm$ 0.06 a |
| Flupyradifurone  | 3.5 $\pm$ 1.94 abc                           | 5 $\pm$ 1.47 ab     | 6 $\pm$ 0.71 ab    | 20.89 $\pm$ 2.15 bc  | 0.56 $\pm$ 0.04 a |
| Spirotetramat    | 1.25 $\pm$ 0.95 bc                           | 1.25 $\pm$ 0.95 c   | 5.5 $\pm$ 1.32 ab  | 19.13 $\pm$ 2.30 cd  | 0.63 $\pm$ 0.04 a |
| Sulfoxaflor      | 3.75 $\pm$ 2.39 ab                           | 3.5 $\pm$ 1.32 bc   | 4.25 $\pm$ 1.65 b  | 22.43 $\pm$ 2.63 abc | 0.54 $\pm$ 0.04 a |

Different letters in each column indicate significant differences between mean values, compared using the Tukey test (5% significance level).

the buckwheat nectar of plants watered with dimethoate and spirotetramat solutions was significantly lower than that in the control treatment ( $\chi^2 = 63.5$ , df = 6,  $p < 0.001$ ) (Table 3). The sex ratios of progeny in the different treatments did not differ ( $\chi^2 = 4.02$ , df = 6,  $p = 0.67$ ).

#### 4. Discussion

Our results show that some of the tested insecticides caused lethal or sublethal effects on *A. vladimiri* via direct pesticide consumption or indirect pesticide consumption through the nectar of treated plants. *Anagyrus vladimiri* females that fed on pesticide solutions containing acetamiprid, dimethoate, flupyradifurone or sulfoxaflor had mortality rates over 60%. Of these four pesticides, all but acetamiprid (dimethoate, flupyradifurone and sulfoxaflor) significantly impacted the survival of *A. vladimiri* females after 3h of feeding. The neonicotinoid acetamiprid has been reported to harm the citrus mealybug parasitoid *Leptomastix dactylopii* Howard (Hymenoptera: Encyrtidae) (Cloyd and Dickinson, 2006). Dimethoate, flupyradifurone and sulfoxaflor have also been shown to cause side effects in other parasitoid species that come into contact with the pesticides or their residues (Brunner et al., 2001; Brar et al., 2017; Tabebordbar et al., 2020). In our study, flupyradifurone was the only tested pesticide that did not affect the Progeny of female parasitoids. Our results also show that cyantraniliprole had a delayed toxic effect on *A. vladimiri*, since survival was not affected immediately but was reduced to half within 2 d after feeding. In fact, cyantraniliprole was the only treatment that reduced the longevity of surviving *A. vladimiri* females a few days after they fed on the pesticide solutions. Cyantraniliprole also led to reduced progeny in parasitoid females; this pesticide has been reported as toxic to other parasitoids, and its compatibility is therefore limited with natural enemies (Amarasekare et al., 2016; Radrigán-Navarro et al., 2021). Direct ingestion of spirotetramat had no toxic effect on *A. vladimiri* females, since this pesticide is only toxic after it has penetrated plant tissues (Nauen et al., 2008).

When *A. vladimiri* females were fed on the nectar of *F. esculentum* irrigated with pesticides, survival and progeny were affected by some of the tested pesticides. The impacts of the pesticides differed in different

monthly trials, except for flupyradifurone, which was harmless to *A. vladimiri* females in all trials. These different responses could be due to the different weather conditions of each trial. In October, the coldest month, no pesticide impacted parasitoid survival; in the other two months, several pesticides had toxic effects on *A. vladimiri*. Interactions between temperature and pesticide impact have been reported in other studies; pesticide toxicity may reduce or increase with temperature (Musser and Shelton, 2005; Nauen et al., 2008; Abbes et al., 2015). As shown by Abbes et al. (2015), four pesticides presented different toxicity levels on the survival and offspring of the parasitoid *Bracon nigricans* Szépligeti (Hymenoptera: Braconidae) when applied at three different temperatures (25 °C, 35 °C and 45 °C) in laboratory bioassays. Furthermore, post-treatment temperature can also influence the toxicity against a pest, such as in the case of *Diaphorina citri* Kuwayama (Hemiptera: Liviidae), where Boina et al. (2009) observed a positive temperature-dependent toxicity correlations of several pesticides against *D. citri* adults in a range of temperatures between 17 and 37 °C. In our study, acetamiprid and spirotetramat reduced the survival of *A. vladimiri* females in August and September, and spirotetramat also negatively impacted parasitoid progeny. The previously reported side effects of neonicotinoids on the fitness of beneficial arthropods when ingested via nectar were the main reason why this pesticide family was included in the present study. Their use has been restricted or forbidden in some countries (European Commission, 2018a; 2018b). Krischik et al. (2007) report that feeding on the flowers of buckwheat treated with the neonicotinoid imidacloprid leads to reduced survival in *Anagyrus pseudococchi* (Girault) (Hymenoptera: Encyrtidae). Although acetamiprid is considered less harmful than other neonicotinoids (Iwasa et al., 2004), such as imidacloprid or thiamethoxam, it may also impact beneficial arthropods. As mentioned above, spirotetramat was not toxic to *A. vladimiri* in our solutions experiment, but it was toxic to parasitoid females that fed on buckwheat plants watered with a spirotetramat solution. This side effect was not observed in the October trial, probably due to the lower temperatures, since pesticide action is reduced when temperatures decrease (Nauen et al., 2008). The progeny of females fed on plants watered with a dimethoate solution was also reduced, as was their survival in the August trial. In the September trial, nectar ingestion of cyantraniliprole and sulfoxaflor affected the survival of *A. vladimiri* females. The presence of these pesticides in nectar has been reported in a wide range of cultivated and non-cultivated plant species (Jiang et al., 2020; Zioga et al., 2020). The concentration of these products could also be impacted by parameters other than temperature, such as the solubility of the pesticide (Edwards, 1975). These factors could explain the variability in the pesticides' toxic effects among the three months of our trials.

Five of the six systemic pesticides tested in this study caused mortality in *A. vladimiri* that directly ingested pesticide solutions. In addition, four of the tested pesticides reduced females' progeny. Direct ingestion of spirotetramat did not affect the parasitoid, but it impacted the survival and progeny of *A. vladimiri* females when this pesticide was applied to buckwheat plants to simulate pesticide that reaches the ground cover due to drift after it is applied to the tree canopy. Acetamiprid, cyantraniliprole, dimethoate and sulfoxaflor also reduced the survival of parasitoids that fed on treated buckwheat plants. In citrus agroecosystems, *A. vladimiri* maintains the citrus mealybug below the economic threshold. Other *Anagyrus* species might also be affected by these pesticides, such as *A. aberiae*, a parasitoid of *D. aberiae*, or *Anagyrus borrianensis* (Hymenoptera: Encyrtidae), a parasitoid of *Pseudococcus viburni* (Hemiptera: Pseudococcidae) (Soler et al., 2021). Further studies are needed to determine the effects of these pesticides on these natural enemies and on citrus agroecosystems.

The presence of a resident or sown vegetation cover between the rows of trees increases the stability of agroecosystems, minimizes the loss of soil nutrients, preserves the intrinsic fertility of the soil, improves water use, minimizes erosion and strengthens agroecosystem diversity (Teixeira et al., 2011; IOBC/WPRS et al., 2022). Proper management of

these plant covers can contribute to an effective citrus IPM strategy, since they can anticipate the arrival of predators in citrus groves, provide an alternative food source for phytoseiid predators (pollen and other spider mites) and for parasitoids (pollen and nectar), and provide shelter for parasitoids and other predators of crop pests (Silva et al., 2010; Aguilar-Fenollosa et al., 2011; Nicholls and Altieri, 2013). The results of the present study highlight the importance of minimizing the amount of pesticide spray that reaches ground cover vegetation, as this might be an important route of contamination for natural enemies. Efficient application equipment can help prevent pesticide loss on the ground due to runoff and drift. This risk should be considered in the design of pesticide control strategies for newly introduced species in citrus to minimize the impacts of pesticides on the biological control of citrus pests.

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## CRediT authorship contribution statement

**Paula Molina:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **José Miguel Campos-Rivela:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Nuria Agustí:** Writing – review & editing, Writing – original draft, Supervision. **María Teresa Martínez-Ferrer:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

## Declaration of competing interest

This article does not contain any studies with human participants or animals, and formal consent is not required.

The statement of potential conflicts of interests: there is absence of any conflict, with regard to both commercial and financial relations, to organizations or persons that could be involved in some way in the study and to interrelations between co-authors of the paper. This statement means that the authors (Paula Molina, José Miguel Campos-Rivela, Nuria Agustí, María Teresa Martínez-Ferrer) assume full responsibility for a possible conflict of interest.

## Data availability

Data will be made available on request.

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