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Research Article

Impact of irrigation with fipronil-contaminated waters on zucchini plants and their main insect pest, *Aphis gossypii*

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ABSTRACT

Fipronil is an insecticide with low residual activity and high efficacy at low concentrations. Due to its widespread use and long half-life, fipronil can persist during wastewater treatment and is found even in surface waters. When released into the environment, it can accumulate over time and can lead to concentrations in soil and plant tissues that are harmful to both target and non-target organisms. As the reuse of treated wastewater and sewage sludge presents challenges and opportunities for farmers, this study investigates the effects of irrigation with environmentally relevant doses of fipronil on zucchini plants and their main insect pest, *Aphis gossypii*. Traces of fipronil were found in the zucchini leaves, but not in the flowers, after 35-days of cumulative exposure. A decrease in nymph-to-adult survival and a dose-dependent reduction in the fecundity of *A. gossypii* feeding on contaminated host plants was observed. Also, aphids feeding on contaminated host plants exhibited the same mortality rate as the control group when exposed to an acute dose of fipronil. However, when natural pyrethrins were used, there was a significant increase in resistance to this insecticide. Our results demonstrate the potential for fipronil to accumulate in plant tissues and highlight the risk of changes in insecticide susceptibility in insect pests. This suggests a need for a holistic approach to the complex dynamics of wastewater reuse in agriculture.

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Introduction

Fipronil is a phenyl pyrazole systemic insecticide developed by Rhone Poulenc Ag Company (now Bayer Crop Science) between 1985 and 1987 (Colliot et al., 1992). It is one of the most persistent lipophilic and toxic insecticides with contact and stomach action even at low field application rates. Fipronil has a broad spectrum use, a long duration, and low residual activity even at low concentrations and can be used effectively against both chewing and piercing sucking insects (Colliot et al., 1992). Fipronil works by blocking the gamma-aminobutyric acid receptor in insects (Hainzl et al., 1998; Ikeda et al., 2004; Mohapatra et al., 2010; Singh et al., 2021).

The fipronil degradation is highly dependent on soil type, water content and the presence of microorganisms (Ying and Kookana, 2002; Pei et al., 2004; Gunasekara et al., 2007; Bhatt et al., 2023). Metabolites can be formed, the most important of which are fipronil desulfinyl, fipronil sulfone, fipronil sulfide, and fipronil amide. These metabolites, except for fipronil amide, have been reported to be more toxic and persistent than the parent compound (Tingle et al., 2003; Gunasekara et al., 2007; Singh et al., 2021).

Fipronil has been used to protect plants against a wide range of insect pests, including *Thrips tabaci, Diatraea saccharalis, Migdolus fryanus, Atta capiguara*, and *Oryzophagus oryzae* (Ester et al., 1997; Cummings et al., 2006; Gonçalves et al., 2022). Fipronil has been shown to be effective against *Pratylenchus zeae*, a root lesion nematode associated with sugarcane (Kawanobe et al., 2019). This insecticide is also used to control the abundance of termites (Steinbauer and Peveling, 2011) and cockroaches (Kaakeh et al., 1997). Fipronil is still used for flea and tick control on domestic animals, providing ectoparasiticidal activity for about one month (Case et al., 2016), but it is banned in animals destined for human consumption.

As a result of its long persistence and high toxicity, a wider range of non-target species are affected by fipronil in different environments. Despite being strictly regulated worldwide, fipronil affects pollinators, insect predators, parasitoids, and other non-pest insects (Stork, 2018). The most studied non-target organisms affected by fipronil are honeybees (Zaluski et al., 2015; Kairo et al., 2017; Holder et al., 2018), aquatic invertebrates in surface waters, and various species of stream invertebrates, such as *Chironomus dilutus* and *Hyalella azteca* (Weston and Lydy, 2014; Hapke et al., 2016). Compared to its effect on aquatic organisms, fipronil has been shown to be extremely toxic to honeybees and even sub-lethal doses ingested throughout the foraging season can adversely affect colony development and maintenance (Zaluski et al., 2015; Kairo et al., 2017; Holder et al., 2018; Carter et al., 2020).

The European Commission published some restrictions on the use of fipronil (EUR-Lex, 2014) after the European Food Safety Authority had identified a high acute risk to bees (EFSA, 2013), and since 2014 fipronil has been banned on maize and sunflowers in Europe. Even without direct agricultural use, the levels of fipronil (and its main metabolites) continue to increase in the environment due to veterinary use, urban pest control, small area turf care, and indoor cockroach traps (Stork, 2018; Sadaria et al., 2019). Fipronil has been found in various environmental media, including wastewater (Heidler and Halden, 2009), runoff (Gan et al., 2012; Sengupta et al., 2014), and surface water (Stone et al., 2014). For example, it was recently found in treated wastewater in Korea at an approximately concentration 20 ng/L (Kim and Kim, 2024). As to surface water, fipronil was recently found at high concentration (mean: 1.77 µg/L) in river waters from the Amazon basin (Cezarette et al., 2024) and at lower concentrations (0.03 µg/L) in the Aquidauana river (Finoto Viana et al., 2023).

Rapid urbanisation and water scarcity due to climate change are driving the development of sustainable water and fertiliser management in agriculture. Wastewater reuse and sewage sludge application present both challenges and opportunities for farmers, society and communities (Trotta et al., 2024a). Fipronil is persistent during conventional wastewater treatment and can be released to the environment through the recovery of treated wastewater and sewage sludge (Weston and Lydy, 2014; McMahen et al., 2016; Supowit et al., 2016; Sadaria et al., 2017). When present in irrigation water, fipronil and some of its metabolites are taken up by the roots and translocated to all parts of the plant (van der Sluijs et al., 2015). For example, 8 weeks old cabbage plants grown from fipronil-coated seeds showed concentrations of fipronil and its transformation products in their leaves less than or equal to 1 µg/kg (Gols et al., 2020). Pesticide residues can then contaminate consumed food, animal feed, soil, water and insects that feed on the plant (Assadpour et al., 2023; Trotta et al., 2024a). These chemicals accumulate in the environment and enter the food chain, with adverse effects on human health and on a wide range of non-target species.

The massive use of chemical pesticides in agriculture to increase yields is causing pests such as aphids to become resistant to their effects. This problem, together with adverse effects on non-target organisms, is mainly due to the indiscriminate use of persistent pesticides. The development of resistance is a major problem and the list of pesticides and chemicals to which pests have developed resistance is incredibly long (Devine et al., 2001; Siddiqui et al., 2023). For example, laboratory-induced resistance to fipronil has been reported in several insect like *Oxycarenus hyalinipennis, Musca domestica, Plutella xylostella*, and *Sogatella furcifera* (Sayyed and Wright, 2004; Tang et al., 2010; Abbas et al., 2014; Wazir and Shad, 2020, 2022).

Typically, pesticide resistance is the result of prolonged and persistent use of these substances, especially at high sub-lethal doses. Insects can be exposed to various insecticides at concentrations as low as parts per billion through the irrigation of crops with wastewater or the application of sludge to soil. Moreover, the use of fipronil-contaminated wastewater for irrigation is a cause for concern also because fipronil accumulates in the soil (Margenat et al., 2019; Brienza and Garcia-Segura, 2022; Trotta et al., 2024a). If polluted water sources are not adequately managed, prolonged environmental exposure to this insecticide could lead to the development of pesticide resistance (Bras et al., 2022) and, more problematically, cross-resistance to other insecticides used for pest control in agroecosystems, such as pyrethrins.

Natural pyrethrins are common non-systemic insecticides widely used in organic farming against a wide range of insect pests (Isman, 2006). Natural pyrethrins are the most commercially used botanical insecticides that rapidly reduce pest populations and have the longest history of effective use. Pyrethrins have been shown to be effective against many species of aphids, including the cotton aphid *Aphis gossypii* Glover (Kalaitzaki et al., 2015). Pyrethrins affect both the peripheral and central nervous systems of insects by interfering with the function of sodium channels, similar to organochlorine insecticide and pyrethroids (Davies et al., 2007). One reason for the success of this group of insecticides is that the range of products registered for organic use is relatively limited. Also, their adoption may be an environmentally friendly practices to be integrated into a management program (Calvin et al., 2021). Pyrethrins have low mammalian toxicity and, unlike fipronil, are highly biodegradable in soil and water, do not leave toxic residues in plants and do not bioaccumulate in food chains (Jeran et al., 2021). The correct use of pyrethrins in a pest management program, preventing the emergence of resistance, would support organic production and offer reduced risk production practices worldwide.

Aphis gossypii is a recurrent polyphagous pest of many cultivated crops worldwide and is considered the most important pest of cucurbits (Blackman and Eastop, 2007). Its direct feeding on some plant species can cause severe damage to plant tissues (Ng and Perry, 2004), but its main damage is associated with phytovirus transmission (Ebert and Cartwright, 1997). This aphid is still largely controlled by a wide range of insecticides, but it is now one of the most insecticide-resistant aphid species (Mota-Sanchez and Wise, 2020).

Although there are many detailed studies on the risks associated with fipronil contamination in aquatic environments, little is known about its potential effects in agricultural environments. This study examined the impact of irrigating zucchini plants with environmentally relevant doses of fipronil on their main insect pest, *A. gossypii*. Zucchini (*Cucurbita pepo* L.) is the most economically important and widely cultivated species among the Cucurbitaceae family and it is one of the most consumed vegetables worldwide (Andolfo et al., 2017). Zucchini plants can be considered an optimal model system for the study of resistance, as the most commonly used strategies to control aphid infestation have focused primarily on the use of pesticides (Cao et al., 2008; Herron and Wilson, 2017). Although pesticides can effectively reduce aphid populations, their use may increase the spread of viruses transmitted by aphids. This is due to the dispersal effect of infected aphids that survive the pesticide treatment (Yuan and Ullman, 1996; Desbiez and Lecoq, 1997). The research focused on fipronil levels in the plants, as well as the fitness costs and changes in insecticide susceptibility of *A. gossypii* when reared on plants exposed to environmentally relevant doses of fipronil. As aphids feed on both zucchini flowers and leaves, the fipronil content was measured in these tissues. The detection of fipronil in the zucchini flowers is of particular interest because the flowers develop later in the plant's growth cycle (about a month after transplanting) and are edible.

1. Materials and methods

1.1. *Chemicals*

The fipronil used in this study was obtained from the commercial product "Frontline® Spot On Gatti", purchased from Boehringer Ingelheim Animal Health Italia S.p.A. (Milan), with the excipients as non-reactive compounds. The pyrethrins were obtained from a commercial product, "Piretro Compo®Bio", an insecticide for organic farming, purchased from COMPO GmbH (Münster), with the excipients as non-reactive compounds.

To simulate a real-world scenario where wastewater reuse and sewage sludge are common agricultural practices, plants were irrigated with the environmentally relevant concentrations of 100 ng/L (coded as F100) of fipronil, and with a higher concentration of 1000 ng/L (F1000) of fipronil. The F100 and F1000 concentrations were obtained by serial dilution of fipronil stock solutions in distilled water.

For the analysis of fipronil in the zucchini plants, analytical standards of fipronil and the internal standard fipronil-$^{13}C_2{}^{15}N_2$ were purchased from Cymit Quimica S.L. (Spain). Stock solutions were prepared in acetonitrile for fipronil and in methanol for fipronil-$^{13}C_2{}^{15}N_2$, with the fipronil stock solution used to prepare calibration curves through serial dilution.

1.2. *Zucchini plant cultivation*

Zucchini seedlings of the cultivar 'San Pasquale' were used in this study. The seeds were kept for germination on wet cotton disks in sterile Petri dishes at 20 ± 2 °C. After one week, the germinated seeds were sown in pots (14 cm diameter) filled with synthetic soil, consisting of a mixture of peat, perlite, sand and clay (Trotta et al., 2024b). The pots were grown under controlled conditions in a climate chamber at 22 ± 1 °C, 60 % ± 5 % relative humidity (RH) and an 18:6 Light:Dark (L:D) photoperiod until the end of the experiments. The lights used for plant growth consisted of 20 W, 130 lm/W white LED tubes (6500 K) coupled with 36 W, 100 lm/W full-spectrum LED tubes. The seedlings were subsequently watered with water plus 10 mL of 0.3 % NPK (7.5–3–6 + Fe and microelements) nutrient solution PIANTE VERDI (Compo®, Ravenna, Italia). These conditions were maintained throughout the entire duration of the experiments, which lasted 35 days.

1.3. *Insect rearing*

The *A. gossypii* strain used in this experiment was collected from a zucchini field near Potenza, Italy (40°34′N, 15°45′E) in August 2022 (Forlano et al., 2022) and reared in the laboratory on zucchini plants. No chemicals were used on this strain from the time of collection until the start of the experiment. Aphids were reared at room temperature under a 16:8 L:D photoperiod for two years. *A. gossypii* can show a wide range of colour variation, from yellow to dark green, as a response to developmental temperature, crowding conditions and host plant. This aphid can also produce winged morphs in response to nutritional factors and crowding. Dark green apterous parthenogenetic females of *A. gossypii* were used for the following experiments.

To eliminate maternal and grandmaternal effects from aphids, 100 adult virginoparae females were isolated from the aphid culture, placed on a fresh host plant and allowed to reproduce for 24 h in a Binder KBF climatic chamber at 22 ± 1 °C, 75 % ± 5 % RH and an 18:6 L:D photoperiod. The adults were then removed, and the cohort of new-born nymphs was reared on the plant for 7 days, corresponding to the beginning of the adult stage. This procedure was carried out for 2 generations and repeated independently for 3 groups of aphids, resulting in individuals from 3 independent replicates.

1.4. *Experimental design of zucchini irrigation with fipronil*

In the control treatment (Co), zucchini plants were watered with tap water. The two experimental treatments involved adding 100 ng/L of fipronil (F100) and 1000 ng/L of fipronil (F1000) to the tap water. These concentrations were selected to reflect environmentally relevant levels (Sadaria et al., 2017, 2019; Zhang et al., 2023; Kim and Kim, 2024), with the higher concentration used to explore potential degradation pathways. Experimental watering began one week after transplanting the seedlings into pots. At each watering, a single plant was watered with 150 mL of the respective water solution. To ensure consistent soil moisture between 30 % and 90 % of water-holding capacity, nine watering sessions were conducted over the 35-day period. Each plant received a total of 1350 mL of experimental water by the end of the experiment. This resulted in a cumulative exposure of 135 ng of fipronil for plants in the F100 treatment and 1350 ng for those in the F1000 treatment, while control plants received no fipronil.

The experiments were conducted in three separate periods, spaced 7 days apart, resulting in three independent replicates of 7 plants each (21 plants in total per treatment group).

1.5. *Aphid survival and fecundity*

For each treatment, two adult female aphids of the same age (8–9 days old) were independently placed on 21 zucchini plants (7 plants per replicate) in the climatic chamber described above. After a few minutes, the two aphids were enclosed inside a clip cage (2 cm of diameter). The clip cages were placed on the adaxial side of the youngest fully developed leaf of each plant. After 24 h, adults and excess newborn nymphs were removed with a wet paintbrush, leaving only three nymphs per clip cage.

For the survival measures, these three nymphs were monitored to adulthood, with aphid mortality assessed on day 7. The offspring of the experimental adults was then assessed by daily counting and removal of new-born nymphs for 7 days (the same number of days as the pre-reproductive period). Aphid fecundity was estimated as the mean number of nymphs per aphid per day.

1.6. *Acute toxicity bioassays*

We assessed the susceptibility of *A. gossypii* to the insecticides fipronil and pyrethrins at sub-lethal doses. Adult aphids that had completed two generations on control (Co) and on plants treated with the F100 and F1000 doses of fipronil were used. The nymphs produced on days 6 and 7 by the experimental adults used for the fecundity experiment were removed from the clip cages and allowed to become adults on another leaf of the same plant. They were then used for the experiments. The tests were performed according to the leaf dip method described in the Insecticide Resistance Action Committee Susceptibility Test Method No 019 (IRAC, 2024), with some modifications. The solutions used were 1 mg/L of fipronil (F-acute), 1 mg/L of pyrethrins (P-acute), and a control (tap water, TW). The doses of fipronil and pyrethrins were chosen based on previously determined acute lethal doses for *A. gossypii* (data not shown). The two insecticides and the tap water were applied to untreated zucchini disc leaves (3 cm of diameter) by dipping them in the test suspensions for 5 s with gentle agitation. Treated disc leaves were placed with their dorsal side on four layers of wet (saturated with distilled water) filter paper in a Petri dish, and 15 adult female aphids were placed on the surface of each treated leaf. Test and control discs leaves were maintained at 22 °C in the thermostatic chamber described above.

For the insecticide susceptibility, nine experimental treatments were generated by combining high-dose insecticide treatments (TW, F-acute and P-acute) and the water treatment applied to the plants where the aphids developed (Co, F100 and F1000). Mortality was estimated after 48 h as the number of live individuals relative to the initial number of aphids. An aphid was considered alive if it could walk or at least move when gently touched with a soft brush. This experiment used three replicates per treatment, with each replicate consisting of at least 3 disc leaves containing 10–15 individuals. This gives a total of at least 100 individuals per treatment.

1.7. *Plant sample collection, fipronil extraction*

As aphids can feed on both zucchini flowers and leaves, plant sampling included collection of leaves and flowers. At the end of the experiment (35 days after transplanting), leaf and flower samples were collected from three separate plants of each experimental treatment, weighed and lyophilised. The extraction of fipronil from the zucchini's leaves and flowers was performed in triplicates, via QuEChERS extraction and dSPE PSA-C18 clean-up protocol (adapted protocol, Montemurro et al., 2020). In short, 1 g of freeze-dried and milled leaves or flowers was placed in a 50 mL falcon tube and hydrated with 9 mL high-performance liquid chromatography (HPLC) water. The tubes were vortexed for 10 s, shaken for 2 min at 2500 r/min and left to hydrate for 1 h. The 10 mL of acetonitrile and 50 µL of formic acid were added in the tubes, vortexing was repeated and the extraction salts (1 g NaCl and 4 g $MgSO_4$) were added in the tubes. The mixture was instantly shaken to prevent the formation of crystalline agglomerates. Tubes were vortexed for 10 s, shaken for 2 min at 2500 r/min and centrifuged at 4 °C for 10 min at 4000 r/min. The supernatant, containing the organic phase, was transferred into glass tubes, and left overnight at −20 °C for the precipitation of fatty acids and waxes. The following day, the clean-up step involved the transfer of 6 mL of the supernatant into the primary secondary amine (PSA) tubes (150 mg PSA, 150 mg C18, 900 mg

$MgSO_4$) and the mixture was vortexed and shaken, then centrifuged at 4 °C for 5 min at 4000 r/min. On the day of the analysis, 1 mL of the supernatant was spiked with the internal standard mixture at a concentration of 20 µg/L. The sample was evaporated until dryness under nitrogen at room temperature, reconstituted with 1 mL of methanol:water (2:8 (V/V)) solution, and injected for ultra-high performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS) (Waters Corporation, MA, USA) analysis.

The analysis of fipronil in the zucchini extracts was performed using ultraperformance liquid chromatography (UPLC), using a Waters Acquity Binary Solvent Manager system (Waters Corporation, MA, USA) coupled to a 5500 QTRAP (Applied Biosystems, Foster City, CA, USA), a quadrupole linear ion trap tandem mass spectrometer (QqLIT) with a Turbo Ion Spray source. Fipronil was analysed in negative ionization mode (method from Castaño-Trias et al., 2023) using an Acquity BEH C18 column (50 mm × 2.1 mm i.d., 1.7 µm particle size) for chromatographic separation. The mobile phase consisted of (A) ACN and (B) 5 mmol/L ammonium acetate/ammonia (pH = 8). The total run was 3.7 min with a flow rate was 0.6 mL/min. The elution gradient was as follows: initial condition 95 % B; 0–1.5 min 5 %–60 % A, 1.5–2 min 60 %−100 % A, 2–3 min 100 % A, 3–3.2 min return to initial conditions, 3.2–3.7 min equilibration of the column. The column temperature was set at 30 °C and an injection volume was set at 5 µL. The quantification of fipronil analytes in zucchini flowers and leaves, as well as the detection (without possibility for quantification) of its metabolites, were performed by selective reaction monitoring by monitoring two mass transitions between the precursor ion and the most abundant fragment ions for each compound. The one at higher intensity was used for quantification purposes, while the second one was used for confirmation of the compound identification. Data acquisition and processing was performed with Analyst 1.5.1 software.

1.8. *Statistical analysis*

Data on nymph-to-adult survival and on the acute toxicity bioassays were analysed using generalized linear mixed-effects models with binomial error (logit link function). For the nymph-to-adult survival model, "water treatment" was the main factor and "replicate nested in treatment" was the random effect. For the acute toxicity bioassay model, "water treatment" and "insecticide treatment" were the fixed factors together with their interactions, and "replicate" nested within the fixed factors was the random effect. The Shapiro-Wilk test was used to test the normality of the fecundity data and then analysed using a linear mixed-effects model fitted with restricted maximum likelihood, with "water treatment" and "reproductive interval" as a fixed factors and "replicate" nested within the fixed factors as random effect.

A backward procedure was used to sequentially remove non-significant effects, allowing the identification of the most parsimonious model. All insect data were analysed using the statistical software R (R Core Team, 2024) with the packages lme4 (Bates et al., 2015) and lmerTest (Kuznetsova et al., 2017).

2. Results and discussion

The results of this study highlight the critical issue of environmental persistence of fipronil, particularly in agricultural systems where wastewater reuse is, or may become, common practice. While extensive research has documented the risks associated with fipronil contamination in aquatic environments, much less is known about its potential effects in agricultural ecosystems. This study addresses this knowledge gap by investigating the impact of irrigation with environmentally relevant doses of fipronil on zucchini plants and their primary insect pest, *A. gossypii*.

2.1. *Fipronil content in zucchini flowers and leaves*

Two types of zucchini samples, the leaves and the flowers, were analysed for fipronil. For the detection and quantification of fipronil, matrix-match calibration curves for both the leaves and flowers were used, exhibiting very good linearity ($R^2 > 0.9997$), in the range 1–100 µg/L. The limits of detection and quantification for fipronil were 29 and 9 ng/g dw (dry weight) in leaves and 15 and 5 ng/g dw in flowers. Average recoveries of fipronil and standard deviations from the triplicate samples in the two matrices were calculated as 102.4 % ± 3.7 % and 105.4 % ± 5.0 % in leaves and flowers, respectively. The analytical method permitted for the detection of fipronil metabolites as well, and the spectrometry parameters used for the detection of all compounds in zucchini samples are given in Table 1.

Table 1 – Spectrometry parameters used for the detection of fipronil and its metabolites in zucchini samples.

Compound	Retention time (min)	Q1–Q3 transitions	DP (V)	CE (V)	CXP (V)
Fipronil	2.06	435.126 / 330.000	−50.000	−22.000	−15.000
		435.126 / 249.800	−50.000	−38.000	−29.000
Fipronil sulfone	2.13	450.868 / 414.900	−125	−24	−23
		450.868 / 281.900	−125	−36	−17
Fipronil sulfide	2.13	418.900 / 382.900	−130	−20	−19
		418.900 / 261.900	−130	−34	−11
Fipronil desulfinyl	2.08	452.884 / 347.800	−90	−16	−17
		452.884 / 303.800	−90	−44	−11
Fipronil-$^{13}C_2{}^{15}N_2$ (I.S.)	2.05	438.906 / 333.900	−75	−22	−15
		438.906 / 251.900	−75	−36	−11

Q1-Q3: precursor and fragment ions in the first and third quadrupole respectively; DP: declustering potential; CE: collision energy; CXP: collision cell exit potential.

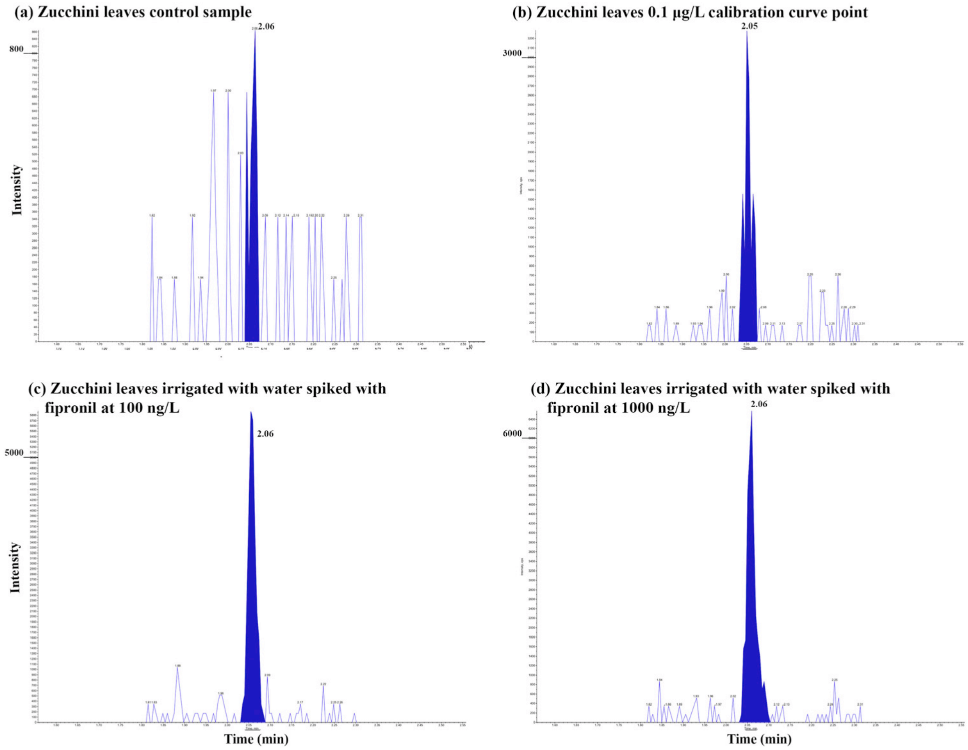
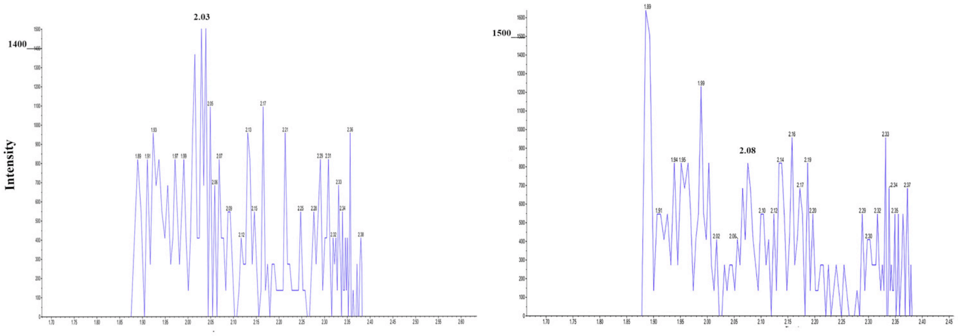


Fig. 1 – Chromatographic peaks of fipronil in the: (a) zucchini leaves control sample (i.e., no spiking); (b) zucchini leaves 0.1 µg/L calibration curve point (lowest curve point); and samples of zucchini irrigated with water spiked with fipronil at (c) 100 ng/L and (d) 1000 ng/L, for zucchini leaves.

Even though fipronil peaks were visible in the chromatograms of the zucchini leaves samples, they were below the detection limit based on the applied calibration curve. Nevertheless, both fragments of the parent ion and the main fragment were identified in those samples. For the leaves, the areas of the peaks in the samples irrigated with the spiked waters were on average 1.3–2× larger compared to the lowest calibration curve point (0.1 µg/L), suggesting some presence of fipronil in the former, albeit in trace amounts. The areas of the samples spiked at 1000 ng/L and at 100 ng/L did not differ, suggesting that at such low levels it would be difficult to differentiate between the two concentrations (Fig. 1). For the flowers, the peaks were either lower than the controls or absent, therefore fipronil was not present in the flower parts of the plant. The method for the detection of fipronil allows for the detection of three of its metabolites as well (fipronil sulfone, fipronil sulfinyl and fipronil desulfinyl), and peaks only for fipronil sulfone (the primary metabolite of fipronil) were detected in the zucchini leaves for both spiked concentrations (Fig. 2); same as with fipronil, both fragments were present, verifying the presence of this metabolite. The area of the fipronil sulfone peak for the sample spiked at 1000 ng/L was on average 1.2× larger than the area of the sample spiked at 100 ng/L.

Fipronil is recognized for its long half-life in soil and water, leading to its accumulation over time and potentially reaching concentrations in soil and plant tissues that may harm both target and non-target organisms (Bhatt et al., 2023). The potential detection of fipronil in zucchini flowers, which develop later in the plant's growth cycle, should further emphasize the widespread contamination and potential risks associated with its use. This aligns with the broader environmental concerns highlighted by the Worldwide Integrated Assessment (WIA) on systemic insecticides, which stress the persistent and pervasive contamination of ecosystems with neonicotinoids and fipronil (van der Sluijs et al., 2015). According to the WIA, these compounds, due to their systemic nature, are absorbed by plants and can persist in the environment for extended periods, leading to chronic exposure of non-target organisms across various ecosystems, including agricultural lands (Pisa et al., 2014). During the experiments, the total volume of water received by the plants was 1.35 L, resulting in a cumulative exposure over 35 days of 135 and 1350 ng fipronil for plants in the F100 and F1000 treatments, respectively. Traces of fipronil were detected in the zucchini leaves, but none were found in the flowers. Moreover, none of the fipronil transformation products included in the analytical protocol (fipronil sulfone, fipronil sulfinyl, and fipronil desulfinyl) was detected in either the leaves or the flowers. It is possible that the total water received by the plants during this short irrigation period was not sufficient for fipronil to accumulate to quantifiable levels in the plant tissues. Prolonged irrigation, which is common in field or greenhouse cultivation, could have resulted in higher levels. It should be remembered that the water uptake of zucchini plants grown under open field conditions is about 250 L/m^2 (Rouphael et al., 2005), depending on the length of the growing cycle (3–5 months). As for the flowers, this experiment cannot determine whether the absence of peaks was due to low levels of fipronil in the


(a) Zucchini leaves control sample

(b) Zucchini leaves 0.1 μg/L calibration curve point

(c) Zucchini leaves irrigated with water spiked with fipronil at 100 ng/L

(d) Zucchini leaves irrigated with water spiked with fipronil at 1000 ng/L

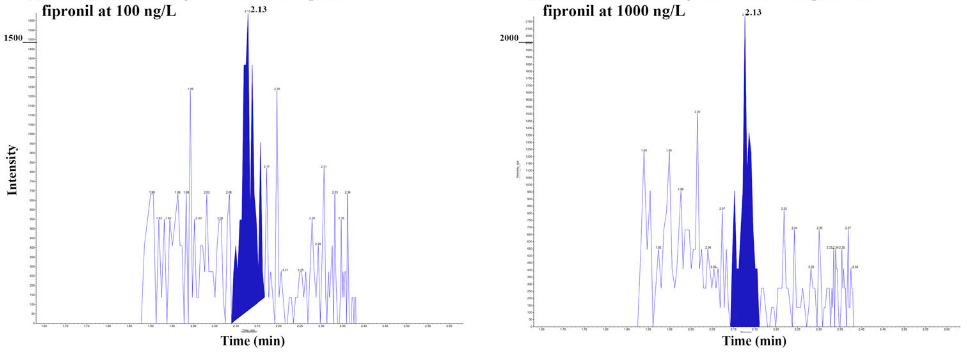


Fig. 2 – Chromatographic peaks of fipronil sulfone in the: (a) zucchini leaves control sample (i.e., no spiking); (b) zucchini leaves 0.1 μg/L calibration curve point (lowest curve point); and samples of zucchini irrigated with water spiked with fipronil at (c) 100 ng/L and (d) 1000 ng/L, for zucchini leaves.

irrigation water or other parts of the plant, or whether it is a mechanistic issue where fipronil is retained only in the leaves and not in the zucchini flowers.

2.2. *Aphid survival and fecundity*

The occurrence of fipronil in plant tissues has direct implications for *A. gossypii*. Aphids engage in a feeding process that involves several short intracellular punctures into parenchyma cells before reaching the phloem. During these punctures, they ingest small amounts of cytoplasm, which contributes to their diet (Alvarez and Griffing, 2023). It is plausible that aphids pick up fipronil through phloematic feeding and also during these brief punctures, which may accumulate in the cytoplasm of the plant cells. This uptake of fipronil present in the host plant, even at low doses, affects the life history traits of *A. gossypii* developed on it.

Nymph-to-adult survival was significantly affected by water treatment ($\chi^2 = 10.01$, d.f. = 2, $P < 0.01$), with F100 and F1000 decreasing aphid survival if compared to the control treatment (Fig. 3).

As expected, the age of the aphids had a significant effect on their fecundity (Fig. 4), with very low values on the first day, peaking around the third day and then gradually decreasing from the fourth day ($F_{6278} = 13.14$, $P < 0.001$). Similar to survival, aphids developed on plants treated with the F100 and F1000 environmental doses of fipronil had a significantly lower fecundity than the control ($F_{2278} = 4.67$, $P < 0.05$). The interaction between the two factors was not significant. The cumulative values fecundity over 7 days of the F1000 treatment (21.8 ± 0.42 nymphs/individual) was lower than that of the F100 (25.8 ± 0.43) and control (27.38 ± 0.44) groups.

In this assay, aphids take up fipronil from the plants watered with environmental doses of the pesticide during their pre-reproductive period (7 days) until the end of the fecundity experiment. Unlike other insecticides such as imidacloprid, fipronil has been shown to cause time-reinforced toxicity in honey bees at trace levels (2.9 ng/bee), resulting in mass mortality of adult individuals (Holder et al., 2018). Fipronil ingested by a honey bee in a single meal was also still present 6 days later (Holder et al., 2018), highlighting the issue of bioaccumulation in living organisms, which can lead to lethal and sub-lethal effects (Zaluski et al., 2015). Our results in aphids are consistent with those found in honeybees and provide evidence of the potentially serious impact of dietary fipronil bioaccumulation on insect life history traits and pest management. Fipronil uptake reduced the fitness of aphids feeding on contaminated host plants, measured as a reduction in nymph-to-adult survival and a dose-dependent reduction in their fecundity. The lowest aphid fecundity observed at the highest dose of fipronil in the irrigation water (1000 ng/L) strongly suggests that the presence and bioaccumulation of the active substance in the mothers diverts resources away from nymph production towards cellular detoxification mechanisms, so that the fecundity of these individuals declines over time. However, this apparent beneficial effect of fipronil at environmental doses for pest control raises the question of adverse effects on human health, the environment, and non-target beneficial organisms, particularly predators and parasitoids commonly used for pest control.

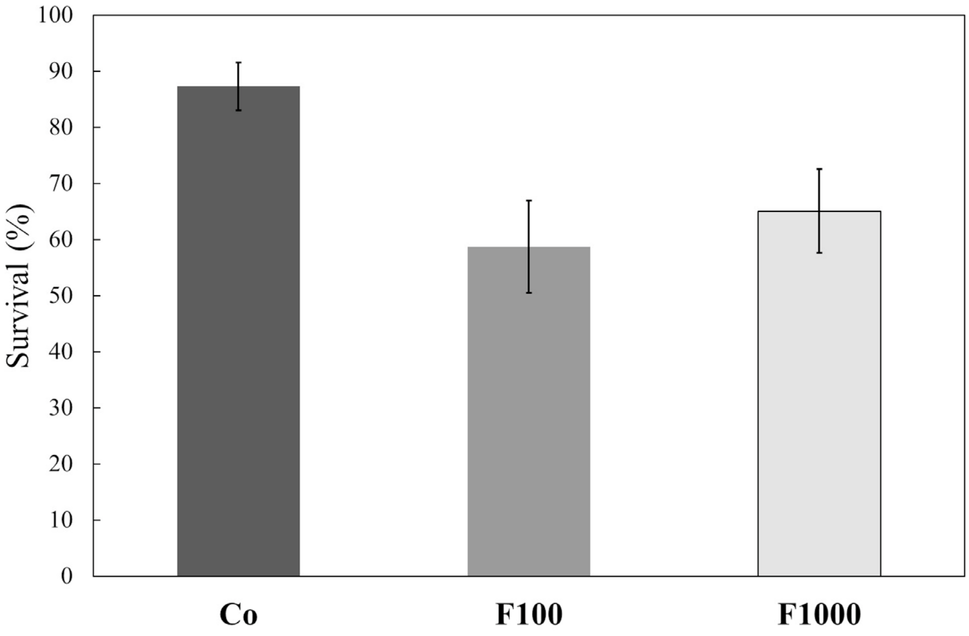


Fig. 3 – Mean percentage of nymph-to adult survival (± standard errors) of *A. gossypii* reared on zucchini plants irrigated with tap water (Co) and with two experimental water treatments obtained by adding 100 ng/L of Fipronil (F100) and 1000 ng/L of Fipronil (F1000) to the irrigation water.

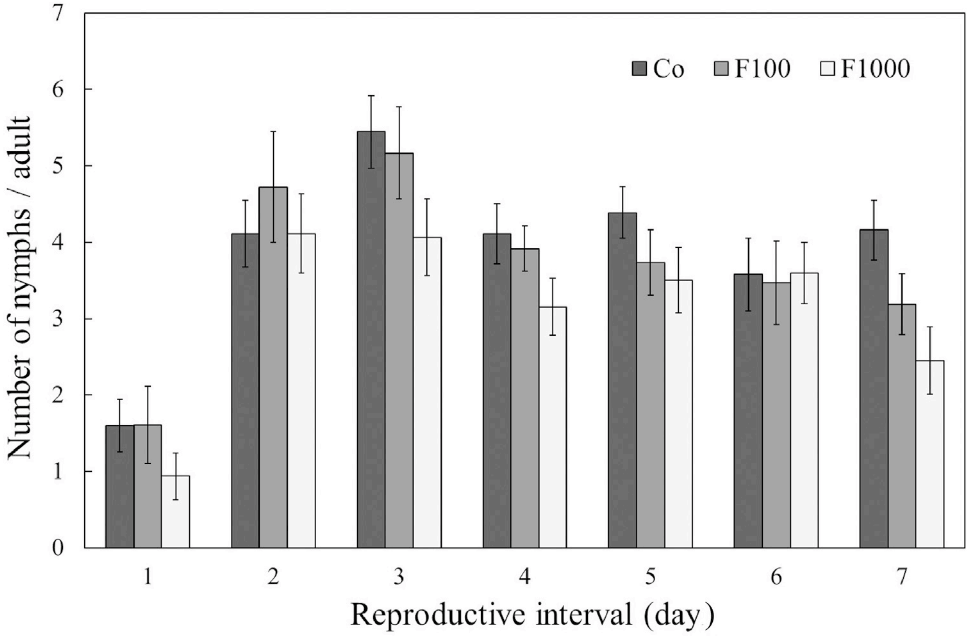


Fig. 4 – Mean percentage of the number of nymphs per aphid per day (± standard errors) of *A. gossypii* reared on zucchini plants irrigated with tap water (Co) and with two experimental water treatments obtained by adding 100 ng/L of Fipronil (F100) and 1000 ng/L of Fipronil (F1000) to the irrigation water.

2.3. *Acute toxicity bioassays*

The environmental persistence of fipronil could potentially lead to the development of resistance in pest populations at realistically low doses of the chemical. The acute toxicity bioassays (Fig. 5) showed that mortality at 48 h of *A. gossypii* was strongly influenced by fipronil and pyrethrins at the concentration of 1 mg/L ($\chi^2 = 298$, d.f. = 2, $P < 0.001$). Fipronil was more effective than pyrethrins against aphids at the same concentration, independently of the water treatment applied to the plants where the aphids developed.

Mortality was not affected by the water treatments ($\chi^2 = 2.4$, d.f. = 2, $P = 0.29$) but, more interestingly, there was a significant interaction between the water treatment and in-

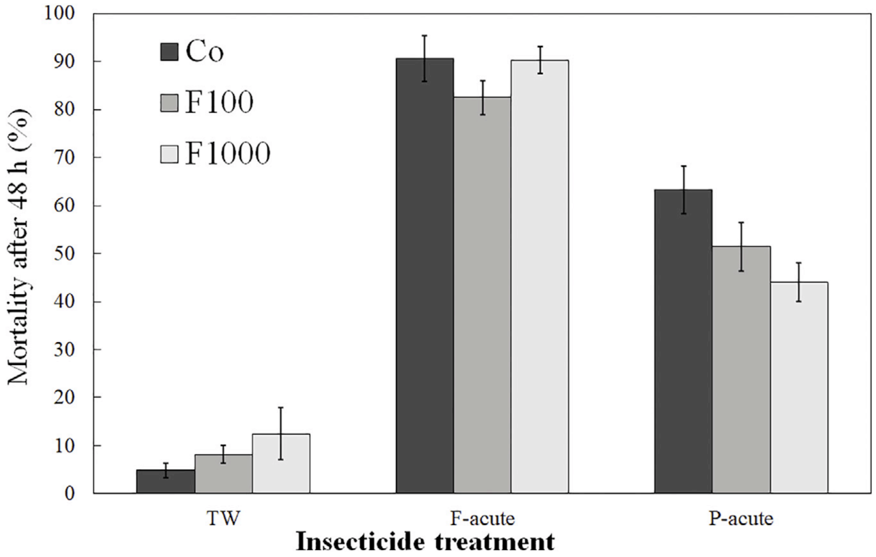


Fig. 5 – Mean percentage of mortality after 48 h (± standard errors) of *A. gossypii* exposed to tap water (TW), to 1 mg/L of fipronil (F-acute), and to 1 mg/L of pyrethrins (P-acute). The two insecticides and the tap water were applied to untreated zucchini disc leaves. Aphids were reared on zucchini plants irrigated with tap water (Co) and with two experimental water treatments obtained by adding 100 ng/L of fipronil (F100) and 1000 ng/L of fipronil (F1000) to the irrigation water.

secticide ($\chi^2 = 12.34$, d.f. = 4, $P < 0.05$), indicating the occurrence of differential susceptibility to a particular insecticide as a function of the presence of fipronil in the plants. Aphids developed on plants irrigated with the F100 or the F1000 doses had the same mortality as the control when exposed to the acute dose of fipronil. When the aphids were exposed to the acute dose of pyrethrins, the mortality of the individuals that developed on the F1000 plants was statistically lower than the control and similar to the F100 (although a decreasing mortality trend was observed).

Laboratory studies of fipronil resistance have been reported in a wide range of insects (Sayyed and Wright, 2004; Tang et al., 2010; Abbas et al., 2014; Wazir and Shad, 2020), suggesting that this insecticide should be applied at high doses where refuges of the insects are located, using chemicals rotation and environmentally friendly cultural practices to delay the development of resistance and prolong its efficacy (Wazir and Shad, 2020, 2022). In the present study, contact acute toxicity bioassays showed no change in aphids mortality when exposed to a sublethal dose of fipronil (1 mg/L), regardless of whether they developed on plants irrigated with environmentally relevant doses of fipronil or on control plants. This result is not surprising, as pesticide resistance is the result of prolonged and persistent use of these substances at sublethal doses and longer experiments might be needed. Aphids that ingest fipronil by feeding on contaminated host plants may activate biomolecular mechanisms that allow them to partially overcome the stress caused by fipronil at sub-lethal doses, as with other abiotic stresses (Hoffmann et al., 2003). However, under our experimental conditions, this effect could have been masked by the high efficacy of fipronil against *A. gossypii* at a concentration of 1 mg/L. When natural pyrethrins were used in the contact acute toxicity bioassays, a significant increase in resistance to pyrethrins was observed in aphids that developed on plants irrigated with fipronil. Our study highlights that if fipronil is not adequately removed from non-conventional irrigation water, prolonged environmental exposure to this insecticide could lead to the development of pesticide resistance and cross-resistance (Bras et al., 2022).

The observed increased resistance to pyrethrins was most likely due to epigenetic mechanisms, as the aphids had only spent two generations on the treated plants, making it unlikely that these changes were due to gene mutation. The rapid development of insecticide resistance in other species, such as the Colorado potato beetle (*Leptinotarsa decemlineata*), which has evolved resistance to over 50 insecticides, illustrates the potential for sublethal insecticide exposure to cause heritable changes due to decreased global DNA methylation (Brevik et al., 2018, 2021). It has been hypothesized that the aphid *Acyrthosiphon pisum* may respond to selective pressure through a transgenerational epigenetic adaptive mechanism that confers plasticity mediated by transposition (De Fabrizio et al., 2024). A similar response has been observed in *Myzus persicae*, where exposure to the insecticide imidacloprid induced reproductive hormesis, accompanied by intermittent changes in the expression of detoxification and stress-coping genes, enabling the insects to better handle subsequent stress (Rix et al., 2016).

3. Conclusions

The current study investigated the persistence and effects of fipronil in an agricultural setting, when using environmentally friendly practices such as unconventional water reuse and a natural, non-persistent insecticide. Our results demonstrate that fipronil had the potential to accumulate in plant tissues, posing risks to non-target organisms. Additionally, the study highlights the risk of fipronil contributing to the development of pesticide resistance and cross-resistance in insect pests like *A. gossypii*. Understanding the ecological impact of fipronil in agro-ecosystems, particularly its role in the emergence of pesticide resistance, shifts in pollinators population dynamics, and effects on other non-target organisms, is crucial. These insights are vital for guiding the implementation of sustainable agricultural practices that minimize environmental contamination and protect biodiversity. However, it is important to highlight the need for detailed biochemical studies to further explore the mechanisms underlying the observed insecticide interactions, as well as the wider ecological implications of the environmental persistence of fipronil. The findings of this study advocate for a more cautious and informed approach to fipronil use in agriculture and emphasize the importance of ongoing monitoring and research to safeguard both ecosystem health and agricultural productivity.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Vittoria Caccavo: Writing – review & editing, Writing – original draft, Supervision, Methodology, Formal analysis. **Monica Brienza:** Writing – review & editing, Validation, Supervision, Resources, Formal analysis, Conceptualization. **Sofia Semitsoglou-Tsiapou:** Writing – review & editing, Validation, Methodology, Formal analysis. **Gianluigi Buttiglieri:** Writing – review & editing, Validation, Supervision, Resources. **Roberto Rosamilia:** Writing – review & editing, Validation, Methodology. **Paolo Fanti:** Writing – review & editing, Validation. **Donatella Battaglia:** Writing – review & editing, Validation, Resources. **Vincenzo Trotta:** Writing – review & editing, Writing – original draft, Validation, Supervision, Methodology, Formal analysis, Data curation, Conceptualization.

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Appendix A Supplementarydata

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.jes.2025.02.005.

REFERENCES

Abbas, N., Khan, H.A.A., Shad, S.A., 2014. Cross-resistance, genetics, and realized heritability of resistance to fipronil in the house fly, *Musca domestica* (Diptera: muscidae): a potential vector for disease transmission. Parasitol. Res. 113, 1343–1352.

Alvarez, A.E., Griffing, L.R., 2023. Are parenchymal cells a source of supplemental diet for aphids? Entomol. Exp. Appl. 171, 449–460.

Andolfo, G., Di Donato, A., Darrudi, R., Errico, A., Cigliano, R.A., Ercolano, M.R., 2017. Draft of Zucchini (*Cucurbita pepo* L.) proteome: a resource for genetic and genomic studies. Front. Genet. 8, 1–5.

Assadpour, E., Can Karaça, A., Fasamanesh, M., Mahdavi, S.A., Shariat-Alavi, M., Feng, J., et al., 2023. Application of essential oils as natural biopesticides; recent advances. Crit. Rev. Food. Sci. Nutr. 64, 6477–6497.

Bates, D., Mächler, M., Bolker, B.M., Walker, S.C., 2015. Fitting linear mixed-effects models using lme4. J. Stat. Softw. 67, 1–48.

Bhatt, P., Gangola, S., Ramola, S., Bilal, M., Bhatt, K., Huang, Y., et al., 2023. Insights into the toxicity and biodegradation of fipronil in contaminated environment. Microbiol. Res. 266, 127247.

Blackman, R.L., Eastop, V.F., 2007. Aphids On the World's Herbaceous Plants and Shrubs. John Wiley & Sons Ltd., Chichester, England West Sussex PO19 8SQ.

Bras, A., Roy, A., Heckel, D.G., Anderson, P., Karlsson Green, K., 2022. Pesticide resistance in arthropods: ecology matters too. Ecol. Lett. 25, 1746–1759.

Brevik, K., Bueno, E.M., McKay, S., Schoville, S.D., Chen, Y.H., 2021. Insecticide exposure affects intergenerational patterns of DNA methylation in the Colorado potato beetle, *Leptinotarsa decemlineata*. Evol. Appl. 14, 746–757.

Brevik, K., Lindström, L., McKay, S.D., Chen, Y.H., 2018. Transgenerational effects of insecticides — Implications for rapid pest evolution in agroecosystems. Curr. Opin. Insect. Sci. 26, 34–40.

Brienza, M., Garcia-Segura, S., 2022. Electrochemical oxidation of fipronil pesticide is effective under environmental relevant concentrations. Chemosphere 307, 135974.

Calvin, W., Beuzelin, J.M., Liburd, O.E., Branham, M.A., Jean Simon, L., 2021. Effects of biological insecticides on the sugarcane aphid, *Melanaphis sacchari* (Zehntner) (Hemiptera: aphididae), in sorghum. Crop Prot. 142, 105528.

Cao, C.W., Zhang, J., Gao, X.W., Liang, P., Guo, H.L., 2008. Overexpression of carboxylesterase gene associated with organophosphorous insecticide resistance in cotton aphids, *Aphis gossypii* (Glover). Pestic. Biochem. Physiol. 90, 175–180.

Carter, L.J., Agatz, A., Kumar, A., Williams, M., 2020. Translocation of pharmaceuticals from wastewater into beehives. Environ. Int. 134, 105248.

Case, K.M., Vega, N.M., Gupta, R.C., Lasher, M.A., Canerdy, T.D., 2016. Safety evaluation of parastar® plus in dogs and assessment of transferable residue of fipronil and cyphenothrin from dogs to humans. Front. Vet. Sci. 3, 89.

Castaño-Trias, M., Rodríguez-Mozaz, S., Buttiglieri, G., 2023. A decade of water monitoring in a Mediterranean region: pharmaceutical prioritisation for an upgraded analytical methodology. Environ. Nanotechnol. Monit. Manag. 20, 100850.

Cezarette, G.N., Souza, M.C.O., Rocha, B.A., González, N., Nadal, M., Domingo, J.L., et al., 2024. Levels and health risk assessment of twenty-one current-use pesticides in urban and riverside waters of the Brazilian Amazon Basin. Environ. Res. 252, 119027.

Colliot, F., Kukorowski, K.A., Hawkins, D.W., Roberts, D.A., 1992. Fipronil: a new soil and foliar broad spectrum insecticide. In: Brighton Crop Protection Conference - Pests and Diseases. Brighton, UK, pp. 29–32.

Cummings, H.D., Brandenburg, R.L., Leidy, R.B., Yelverton, F.H., 2006. Impact of fipronil residues on mole cricket (Orthoptera: Gryllotalpidae) behavior and mortality in bermudagrass. FLA Entomol. 89, 293–298.

Davies, T.G.E., Field, L.M., Usherwood, P.N.R., Williamson, M.S., 2007. DDT, pyrethrins, pyrethroids and insect sodium channels. IUBMB Life 59, 151–162.

De Fabrizio, V., Trotta, V., Pariti, L., Radice, R.P., Martelli, G., 2024. Preliminary characterization of biomolecular processes related to plasticity in *Acyrthosiphon pisum*. Heliyon 10, e23650.

Desbiez, C., Lecoq, H., 1997. Zucchini yellow mosaic virus. Plant. Pathol. 46, 809–829.

Devine, G.J., Barber, M., Denholm, I., 2001. Incidence and inheritance of resistance to METI-acaricides in European strains of the two-spotted spider mite (*Tetranychus urticae*) (Acari: tetranychidae). Pest. Manag. Sci. 57, 443–448.

Ebert, T.A., Cartwright, B.O., 1997. Biology and ecology of *Aphis gossypii* glover (Homoptera: Aphididae). Southwest. Entomolog. 22, 116–153.

EFSA (European Food Safety Authority), 2013. Conclusion on the Peer Review of the Pesticide Risk Assessment for Bees for the Active Substance Fipronil. Available at: https://www.efsa.europa.eu/en/efsajournal/pub/3158. Accessed Aug. 1, 2023.

Ester, A., De Vogel, R., Bouma, E., 1997. Controlling *Thrips tabaci* (Lind.) in leek by film-coating seeds with insecticides. Crop Prot. 16, 673–677.

EUR-Lex (Access to European Union law), 2014. Commission Regulation (EU) No 1127/2014 Of 20 October 2014 Amending Annexes II and III to Regulation (EC) No 396/2005 of the European Parliament and of the Council as Regards Maximum Residue Levels for Amitrole, Dinocap, Fipronil, Flufenacet, Pendimethali. Available at: http://data.europa.eu/eli/reg/2014/1127/oj. Accessed Aug. 1, 2024.

Finoto Viana, L., do Amaral Crispim, B., Kummrow, F., Alice de Lima, N., Amaral Dias, M., Carolina Montagner, C., et al., 2023. Occurrence of contaminants of emerging concern and their risks to the Pantanal Sul-Mato-Grossense aquatic biota, Brazil. Chemosphere 337, 139429.

Forlano, P., Mang, S.M., Caccavo, V., Fanti, P., Camele, I., Battaglia, D., et al., 2022. Effects of below-ground microbial biostimulant *Trichoderma harzianum* on diseases, insect community, and plant performance in *Cucurbita pepo* L. under open field conditions. Microorganisms 10, 2242.

Gan, J., Bondarenko, S., Oki, L., Haver, D., Li, J.X., 2012. Occurrence of fipronil and its biologically active derivatives in urban residential runoff. Environ. Sci. Technol. 46, 1489–1495.

Gols, R., WallisDeVries, M.F., van Loon, J.J.A., 2020. Reprotoxic effects of the systemic insecticide fipronil on the butterfly *Pieris brassicae*. Proc. R. Soc. B 287, 20192665.

Gonçalves, S., Vasconcelos, M.W., Mota, T.F.M., Lopes, J.M.H., Guimaraes, L.J., Miglioranza, K.S.B., et al., 2022. Identifying global trends and gaps in research on pesticide fipronil: a scientometric review. Environ. Sci. Pollut. Res. 29, 79111–79125.

Gunasekara, A.S., Truong, T., Goh, K.S., Spurlock, F., Tjeerdema, R.S., 2007. Environmental fate and toxicology of fipronil. J. Pestic. Sci. 32, 189–199.

Hainzl, D., Cole, L.M., Casida, J.E., 1998. Mechanisms for selective toxicity of fipronil insecticide and its sulfone metabolite and desulfinyl photoproduct. Chem. Res. Toxicol. 11, 1529–1535.

Hapke, W.B., Morace, J.L., Nilsen, E.B., Alvarez, D.A., Masterson, K., 2016. Year-round monitoring of contaminants in Neal and Rogers Creeks, Hood River basin, Oregon, 2011-12, and assessment of risks to salmonids. PLoS One 11, e0158175.

Heidler, J., Halden, R.U., 2009. Fate of organohalogens in US wastewater treatment plants and estimated chemical releases to soils nationwide from biosolids recycling. J. Environ. Monit. 11, 2207–2215.

Herron, G.A., Wilson, L.J., 2017. Can resistance management strategies recover insecticide susceptibility in pests?: a case study with cotton aphid *Aphis gossypii* (Aphididae: Hemiptera) in Australian cotton. Aust. Entomol. 56, 1–13.

Hoffmann, A.A., Sørensen, J.G., Loeschcke, V., 2003. Adaptation of *Drosophila* to temperature extremes: bringing together quantitative and molecular approaches. J. Therm. Biol. 28, 175–216.

Holder, P.J., Jones, A., Tyler, C.R., Cresswell, J.E., 2018. Fipronil pesticide as a suspect in historical mass mortalities of honey bees. Proc. Natl. Acad. Sci. U.S.A. 115 (51), 13033–13038.

Ikeda, T., Nagata, K., Kono, Y., Yeh, J.Z., Narahashi, T., 2004. Fipronil modulation of GABAA receptor single-channel currents. Pest Manag. Sci. 60, 487–492.

Isman, M.B., 2006. Botanical insecticides, deterrents, and repellents in modern agriculture and an increasingly regulated world. Annu. Rev. Entomol. 51, 45–66.

Jeran, N., Grdiša, M., Varga, F., Šatović, Z., Liber, Z., Dabić, D., et al., 2021. Pyrethrin from Dalmatian pyrethrum (*Tanacetum cinerariifolium* (Trevir.) Sch. Bip.): biosynthesis, biological activity, methods of extraction and determination. Phytochem. Rev. 20, 875–905.

Kaakeh, W., Reid, B.L., Bennett, G.W., 1997. Toxicity of fipronil to German and American cockroaches. Entomol. Exp. Appl. 84, 229–237.

Kairo, G., Poquet, Y., Haji, H., Tchamitchian, S., Cousin, M., Bonnet, M., et al., 2017. Assessment of the toxic effect of pesticides on honey bee drone fertility using laboratory and semifield approaches: a case study of fipronil. Environ. Toxicol. Chem. 36, 2345–2351.

Kalaitzaki, A., Papanikolaou, N.E., Karamaouna, F., Dourtoglou, V., Xenakis, A., Papadimitriou, V., 2015. Biocompatible colloidal dispersions as potential formulations of natural pyrethrins: a structural and efficacy study. Langmuir 31, 5722–5730.

Kawanobe, M., Miyamaru, N., Yoshida, K., Kawanaka, T., Fujita, T., Toyota, K., 2019. Sugarcane yield loss in the ratoon crop carried over from the plant crop damaged by plant-parasitic nematode in a heavy clay field in Okinawa, Japan. Nematol. Res. 49, 1–6.

Kim, H., Kim, S.D., 2024. Pesticides in wastewater treatment plant effluents in the Yeongsan River Basin, Korea: occurrence and environmental risk assessment. Sci. Total Environ. 946, 174388.

Kuznetsova, A., Brockhoff, P.B., Christensen, R.H.B., 2017. lmerTest Package: tests in Linear Mixed Effects Models. J. Stat. Softw. 82, 1–26.

Margenat, A., Matamoros, V., Díez, S., Cañameras, N., Comas, J., Bayona, J.M., 2019. Occurrence and human health implications of chemical contaminants in vegetables grown in peri-urban agriculture. Environ. Int. 124, 49–57.

McMahen, R.L., Strynar, M.J., McMillan, L., DeRose, E., Lindstrom, A.B., 2016. Comparison of fipronil sources in North Carolina surface water and identification of a novel fipronil transformation product in recycled wastewater. Sci. Total Environ. 569–570, 880–887.

Mohapatra, S., Deepa, M., Jagdish, G.K., Rashmi, N., Kumar, S., Prakash, G.S., 2010. Fate of fipronil and its metabolites in/on grape leaves, berries and soil under semi arid tropical climatic conditions. Bull. Environ. Contam. Toxicol. 84, 587–591.

Montemurro, N., Orfanioti, A., Manasfi, R., Thomaidis, N.S., Pérez, S., 2020. Comparison of high resolution mrm and sequential window acquisition of all theoretical fragment-ion acquisition modes for the quantitation of 48 wastewater-borne pollutants in lettuce. J. Chromatogr. A 1631, 461566.

Mota-Sanchez, D., Wise, J.C., 2020. The Arthropod Pesticide Resistance Database. Michigan State University. Available at: https://www.pesticideresistance.org/index.php. Accessed Aug. 1, 2024

Ng, J.C.K., Perry, K.L., 2004. Transmission of plant viruses by aphid vectors. Mol. Plant Pathol. 5, 505–511.

Pei, Z., Yitong, L., Baofeng, L., Gan, J.J., 2004. Dynamics of fipronil residue in vegetable-field ecosystem. Chemosphere 57, 1691–1696.

Pisa, L.W., Amaral-Rogers, V., Belzunces, L.P., Bonmatin, J.-M., Downs, C.A., Goulson, D., 2014. Effects of neonicotinoids and fipronil on non-target invertebrates. Environ. Sci. Pollut. Res. 22, 68–102.

R Core Team, 2024. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.. Available at: https://www.R-project.org/. Accessed June 1, 2024.

Rix, R.R., Ayyanath, M.M., Cutler, G.C., 2016. Sublethal concentrations of imidacloprid increase reproduction, alter expression of detoxification genes, and prime *Myzus persicae* for subsequent stress. J. Pest. Sci. 89, 581–589.

Rouphael, Y., Colla, G., Cardarelli, M., Fanasca, S., Salerno, A., Rivera, C.M., et al., 2005. Water use efficiency of greenhouse summer squash in relation to the method of culture: soil vs. soilless. Acta Hortic. 697, 81–86.

Sadaria, A.M., Labban, C.W., Steele, J.C., Maurer, M.M., Halden, R.U., 2019. Retrospective nationwide occurrence of fipronil and its degradates in U.S. wastewater and sewage sludge from 2001 - 2016. Water Res. 155, 465–473.

Sadaria, A.M., Sutton, R., Moran, K.D., Teerlink, J., Brown, J.V., Halden, R.U., 2017. Passage of fiproles and imidacloprid from urban pest control uses through wastewater treatment plants in northern California, USA. Environ. Toxicol. Chem. 36, 1473–1482.

Sayyed, A.H., Wright, D.J., 2004. Fipronil resistance in the diamondback moth (Lepidoptera: plutellidae): inheritance and number of genes involved. J. Econ. Entomol. 97, 2043–2050.

Sengupta, A., Lyons, J.M., Smith, D.J., Drewes, J.E., Snyder, S.A., Heil, A., et al., 2014. The occurrence and fate of chemicals of emerging concern in coastal urban rivers receiving discharge of treated municipal wastewater effluent. Environ. Toxicol. Chem. 33, 350–358.

Siddiqui, J.A., Fan, R., Naz, H., Bamisile, B.S., Hafeez, M., Ghani, M.I., et al., 2023. Insights into insecticide-resistance mechanisms in invasive species: challenges and control strategies. Front. Physiol. 13, 1–18.

Singh, N.S., Sharma, R., Singh, S.K., Singh, D.K., 2021. A comprehensive review of environmental fate and degradation of fipronil and its toxic metabolites. Environ. Res. 199, 111316.

Steinbauer, M.J., Peveling, R., 2011. The impact of the locust control insecticide fipronil on termites and ants in two contrasting habitats in northern Australia. Crop Prot. 30, 814–825.

Stone, W.W., Gilliom, R.J., Ryberg, K.R., 2014. Pesticides in U.S. streams and rivers: occurrence and trends during 1992-2011. Environ. Sci. Technol. 48, 11025–11030.

Stork, N.E., 2018. How many species of insects and other terrestrial arthropods are there on earth? Annu. Rev. Entomol. 63, 31–45.

Supowit, S.D., Sadaria, A.M., Reyes, E.J., Halden, R.U., 2016. Mass balance of fipronil and total toxicity of fipronil-related compounds in process streams during conventional wastewater and wetland treatment. Environ. Sci. Technol. 50, 1519–1526.

Tang, J., Li, J., Shao, Y., Yang, B., Liu, Z., 2010. Fipronil resistance in the whitebacked planthopper (*Sogatella furcifera*): possible resistance mechanisms and cross-resistance. Pest Manag. Sci. 66, 121–125.

Tingle, C.C.D., Rother, J.A., Dewhurst, C.F., Lauer, S., King, W.J., 2003. Fipronil: environmental fate, ecotoxicology, and human health concerns. In: Ware, G.W. (Ed.), BT - Reviews of Environmental Contamination and Toxicology: Continuation of Residue Reviews. Springer, New York, pp. 1–66.

Trotta, V., Baaloudj, O., Brienza, M., 2024a. Risks associated with wastewater reuse in agriculture: investigating the effects of contaminants in soil, plants, and insects. Front. Environ. Sci. 12, 1–7.

Trotta, V., Russo, D., Rivelli, A.R., Battaglia, D., Bufo, S.A., Caccavo, V., et al., 2024b. Wastewater irrigation and *Trichoderma* colonization in tomato plants: effects on plant traits, antioxidant activity, and performance of the insect pest *Macrosiphum euphorbiae*. Environ. Sci. Pollut. Res. 31, 18887–18899.

van der Sluijs, J.P., Amaral-Rogers, V., Belzunces, L.P., Bijleveld van Lexmond, M.F.I.J., Bonmatin, J.-M., Chagnon, M., et al., 2015. Conclusions of the Worldwide Integrated Assessment on the risks of neonicotinoids and fipronil to biodiversity and ecosystem functioning. Environ. Sci. Pollut. Res. 22, 148–154.

Wazir, S., Shad, S.A., 2020. Inheritance mode and properties of fipronil resistance in *Oxycarenus hyalinipennis* Costa (Hemiptera: Lygaeidae). J. Asia Pac. Entomol. 23, 1055–1061.

Wazir, S., Shad, S.A., 2022. Development of fipronil resistance, fitness cost, cross-resistance to other insecticides, stability, and risk assessment in *Oxycarenus hyalinipennis* (Costa). Sci. Total Environ. 803, 150026.

Weston, D.P., Lydy, M.J., 2014. Toxicity of the insecticide fipronil and its degradates to benthic macroinvertebrates of urban streams. Environ. Sci. Technol. 48, 1290–1297.

Ying, G.-G., Kookana, R., 2002. Laboratory and field studies on the degradation of fipronil in a soil. Soil Res. 40, 1095.

Yuan, C., Ullman, D.E., 1996. Comparison of efficiency and propensity as measures of vector importance in zucchini yellow mosaic potyvirus transmission by *Aphis gossypii* and *A. craccivora*. Phytopathology 86, 698–703.

Zaluski, R., Kadri, S.M., Alonso, D.P., et al., 2015. Fipronil promotes motor and behavioral changes in honey bees (*Apis mellifera*) and affects the development of colonies exposed to sublethal doses. Environ. Toxicol. Chem. 34, 1062–1069.

Zhang, Q., Yang, Y., Xiao, Y., et al., 2023. Identification, occurrence, concentration and composition profile of fiproles in municipal wastewater treatment plants. Sci. Total Environ. 888, 164198.

IRAC (International Regulatory Approval Committee), 2024. Insecticide Resistance Action Committee - Test Method Library. Available at: https://irac-online.org/test-methods/test-method-library/. Accessed Jul. 1, 2024