IPM-recommended insecticides harm beneficial insects through contaminated honeydew☆

Miguel Calvo-Agudo [a, d, *], Joel González-Cabrera [b], Daniele Sadutto [c], Yolanda Picó [c], Alberto Urbaneja [a], Marcel Dicke [d], Alejandro Tena [a]

[a] Instituto Valenciano de Investigaciones Agrarias (IVIA), Centro de Protección Vegetal y Biotecnología, Unidad Mixta Gestión Biotecnológica de Plagas UV-IVIA, Carretera CV-315, Km 10,7, 46113, Moncada, Spain
[b] Universitat de València, Estructura de Recerca Interdisciplinar en Biotecnologia i Biomedicina (ERI-BIOTECMED), Unidad Mixta Gestión Biotecnológica de Plagas UV-IVIA, Dr Moliner 50, 46100, Burjassot, Spain
[c] Universitat de València, Centre of Research on Desertification (CIDE, CSIC-GV-UV), Carretera CV-315, Km 10,7, 46113, Moncada, Spain
[d] Wageningen University, Laboratory of Entomology, PO Box 16, 6700AA, Wageningen, the Netherlands

ARTICLE INFO

ABSTRACT

The use of some systemic insecticides has been banned in Europe because they are toxic to beneficial insects when these feed on nectar. A recent study shows that systemic insecticides can also kill beneficial insects when they feed on honeydew. Honeydew is the sugar-rich excretion of hemipterans and is the most abundant carbohydrate source for beneficial insects such as pollinators and biological control agents in agroecosystems. Here, we investigated whether the toxicity of contaminated honeydew depends on i) the hemipteran species that excretes the honeydew; ii) the active ingredient, and iii) the beneficial insect that feeds on it. HPLC-MS/MS analyses demonstrated that the systemic insecticides pymetrozine and flonicamid, which are commonly used in Integrated Pest Management programs, were present in honeydew excreted by the mealybug *Planococcus citri*. However, only pymetrozine was detected in honeydew excreted by the whitefly *Aleurothixus floccosus*. Toxicological studies demonstrated that honeydew excreted by mealybugs feeding on trees treated either with flonicamid or pymetrozine increased the mortality of the hoverfly *Sphaerophoria rueppellii*, but did not affect the parasitic wasp *Anagyrus vladimiri*. Honeydew contaminated with flonicamid was more toxic for the hoverfly than that contaminated with pymetrozine. Collectively, our data demonstrate that systemic insecticides commonly used in IPM programs can contaminate honeydew and kill beneficial insects that feed on it, with their toxicity being dependent on the active ingredient and hemipteran species that excretes the honeydew.
© 2020 The Author(s). Published by Elsevier Ltd. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

1. Introduction

Systemic insecticides are widely used to manage insect pests in agriculture. However, these insecticides can impact non-target beneficial insects directly through contact when they are sprayed in crops, and indirectly through the food chain via cascading effects (Desneux et al., 2007; Kampfraath et al., 2017). One of the best-known routes of indirect exposure of systemic insecticides to beneficial insects is through the contamination of plant-derived food sources such as nectar and pollen (Bonmatin et al., 2015).

For instance, the most widely used systemic insecticides, i.e. neonicotinoids, are well-known to reach these plant-derived food sources at concentrations ranging from 0.7 to 100 μg/kg (Bonmatin et al., 2015; Whitehorn et al., 2012). A vast majority of beneficial insects, which provide ecosystem services like pollination or pest control (Losey and Vaughan, 2006), are highly dependent on these plant-derived food sources to support their daily physical activities and metabolic processes (Lundgren, 2009). As a consequence, a plethora of beneficial insects are exposed to lethal or sublethal concentrations of neonicotinoids when they feed on pollen and nectar. Their overuse has been considered one of the main stressors implicated in the decline of some pollinators (Henry et al., 2012; Sánchez-Bayo et al., 2017; Stapel et al., 2000; Tappert et al., 2017). For this reason, the use of several neonicotinoids was banned in Europe (European Food Safety Authority, 2018). However, there are other systemic insecticides that are still widely used. For example,

the phloem-transported insecticides flonicamid and pymetrozine are applied against numerous pests in many crops. These insecticides have different modes of action, but ultimately both disrupt feeding and other behaviors in target insects (Belchim, 2020; Syngenta, 2020). Pymetrozine binds to and disrupts the gating properties of Nan-Iav TRPV (Transient Receptor Potential Vanilloid) channel complexes in chordotonal stretch receptor organs. It induces, among other, neural inhibition of feeding behavior that eventually starves insects (Group 9B; Insecticide Resistance Action Committee, 2020). Flonicamid is also a modulator of the chordotonal organ function, but the specific site(s) responsible for its biological activity is still unknown. It is believed that it disturbs the insect feeding patterns (Group 29; Insecticide Resistance Action Committee, 2020). Both pymetrozine and flonicamid are considered selective and less toxic to beneficial insects than neonicotinoids. Therefore, they are recommended in different Integrated Pest Management (IPM) programs (Jansen et al., 2011).

Many ecotoxicological studies have evaluated the toxicity of these IPM-recommended insecticides on beneficial insects (Barbosa et al., 2018; Colomer et al., 2011; Jansen et al., 2011; Joseph et al., 2011; Moens et al., 2011; Tran et al., 2004). However, none of these studies have analysed a route of exposure that has been recently described, i.e. contaminated honeydew (Calvo-Agudo et al., 2019). Honeydew is the sugar-rich excretion product of hemipteran phloem feeders such as aphids, coccids, whiteflies, and psyllids that feed on crops, weeds or the surrounding natural vegetation (Heimpel and Jervis, 2005; Wäckers et al., 2005). Honeydew has typically been overlooked as a food source for beneficial insects because it was considered a carbohydrate source of poorer quality than nectar (Downes and Dahlem, 1987; Hagen, 1962; Lundgren, 2009; Wäckers et al., 2008). However, its quality as carbohydrate source for beneficial insects is variable and, due to its high degree of accessibility and abundance, it is the main carbohydrate source in most agroecosystems (Lundgren, 2009; Tena et al., 2016). In fact, honeydew is exploited by many beneficial insects including bees, hoverflies, ants, parasitic wasps and predators (Calabuig et al., 2015; Cameron et al., 2019; Hogervorst et al., 2007; Hölldobler and Wilson, 1990; Konrad et al., 2009; Lee et al., 2006; Tena et al., 2013b), likely because honeydew is more abundant than nectar and pollen in many agroecosystems (Lundgren, 2009; Tena et al., 2016; Wäckers et al., 2008).

It has been recently demonstrated that the systemic insecticides thiamethoxam, imidacloprid and spirotetramat are detected in honeydew excreted by hemipterans feeding on plants treated with these insecticides (Calvo-Agudo et al., 2019; Quesada et al., 2020). Furthermore, honeydew excreted by mealybugs feeding on trees treated with thiamethoxam or imidacloprid can be toxic for the pollinator and predator hoverfly *Sphaerophoria rueppellii* (Wiedemann) (Diptera: Syrphidae) and the parasitic wasp *Anagyrus vladimiri* (Girault) (Hymenoptera: Encyrtidae) (previously known as *A. pseudococci*) (Calvo-Agudo et al., 2019). Here, we explored whether the IPM-recommended insecticides pymetrozine and flonicamid: i) reach honeydew under controlled and field conditions when hemipterans feed on treated plants; ii) have lethal and/or sublethal effects on beneficial insects that feed on it; and iii) whether the excretion of insecticides differs between hemipteran species.

2. Materials and methods

2.1. System

We selected citrus as crop because numerous honeydew-producing species feed on citrus trees. Among the honeydew producers, the mealybug *Planococcus citri* (Risso) (Hemiptera: Pseudococcidae) was selected because: it is common in many citrus producing areas of the world although hardly ever reaching the economic injury level (Urbaneja et al., 2020); it excretes honeydew that increases the longevity and fecundity of beneficial insects (Tena et al., 2013a); and mealybugs are known to be tolerant to the insecticides pymetrozine and flonicamid (El-Zahi et al., 2016; Rezk et al., 2019). As beneficial insects, we selected the hoverfly *S. rueppellii* and the parasitic wasp *A. vladimiri*. *Sphaerophoria rueppellii* was selected because hoverflies are one of the most important groups of pollinators (Rader et al., 2015), their larvae feed on aphids and their populations are declining (Powney et al., 2019). *Anagyrus vladimiri* was selected because parasitic wasps represent one of the main groups of biological control agents in agriculture (Heimpel and Mills, 2017); it is the main biological control agent of *P. citri* and the genus *Anagyrus* represents one of the most successful examples used in biological control worldwide (Herren and Neuenschwander, 1991).

2.2. Insects and experimental conditions

The phloem-feeding herbivorous insect *P. citri* was obtained from the State Insectary of Generalitat Valenciana (Almassora, Spain), where it was reared on potato sprouts and transported to the Instituto Valenciano de Investigaciones Agrarias (IVIA) (Moncada, Spain) as crawlers (first instar) (Planes et al., 2013). The parasitic wasp *A. vladimiri* and the predator-pollinator *S. rueppellii* were obtained as pupae from the commercial companies Koppert Biological Systems S.L (Águilas, Spain) and Biobest Biological Systems (Westerlo, Belgium), respectively. Pupae were introduced into wooden and glass rearing boxes ($51 \times 51 \times 41$ cm) with holes in the wall that were covered with anti-aphid mesh. Rearing boxes were kept in the laboratory at room temperature until adults emerged. Unfed newly emerged parasitic wasps and hoverflies were collected daily between 9:00 and 11:00 a.m. and used in the experiments. All experiments were carried out in different climatic chambers for each insect species at 25 ± 2 °C, $75 \pm 10\%$ RH and a photoperiod of 14:10 h (L:D).

2.3. Plant infestation and insecticide application

2.3.1. Under controlled conditions

Twenty-seven potted clementine trees cv. Clementina de Nules grafted on 'Macrophyla' (*Citrus sinensis* × *Poncirus trifoliata*) were grown in a greenhouse at IVIA until they were one-year-old and ~1 m high. The environmental conditions in the greenhouse compartments were 22 ± 5 °C, $70 \pm 20\%$ RH and natural photoperiod (February−April 2017). Clementine trees were watered three times per week and fertilized once per week with Sofertirrig® fertilizer (18-18-18 N-P-K). Plants were infested with *P. citri* crawlers on March 7, 2017. To infest them, 1.5 mL centrifuge tubes half-filled with *P. citri* crawlers were held on the crown of each plant (Calvo-Agudo et al., 2019). On 26 April 2017, we applied each insecticide or distilled water (control treatment) in separate chambers to nine clementine plants per treatment that we temporally removed from the greenhouse in order to prevent spray drift and cross-contamination of treatments. The insecticides used in this research were flonicamid [Flonicamid (50%), Teppeki WG, Belchim] and pymetrozine [(Pymetrozine (50%), Plenum WG, Syngenta)]. Insecticides were sprayed at the dose recommended by the manufacturer. A concentration of 0.05 g of flonicamid/L of distilled water and a concentration of 0.4 g of pymetrozine/L of distilled water were applied on nine different plants per treatment. Water-treated trees (controls) were sprayed using only distilled

water. We used 2 L manual sprayers and a different sprayer was used for each insecticide and the control. Insecticides were sprayed until run-off (200 mL per tree). One hour after spraying, the trees were returned to their previous positions in the greenhouse.

2.3.2. Under field conditions

Twelve 20-year-old untreated orange trees (*Citrus sinensis*) located at the Instituto Valenciano de Investigaciones Agrarias (UTM: 39°35′16.4″N 0°23′54.2″W) were selected and infested with *P. citri* crawlers on 20 August, 2018. Trees were approximately 2.5 m high. One twig per tree was infested. To infest the twigs, 1.5 mL centrifuge tubes half-filled with *P. citri* crawlers were held on the twig and covered individually with sleeve bags made from fine mesh organdy to allow ventilation and prevent *P. citri* crawlers from escaping. Mealybugs were kept undisturbed within the sleeve bags for 21 days. On 11 September 2018, we removed the exclusion bags and applied the insecticides flonicamid or pymetrozine or distilled water as control treatment. At this period of the year, the whitefly *Aleurothrixus floccosus* Maskell (Hemiptera: Aleyrodidae) had naturally infested all the selected trees. Two whitefly colonies were selected per tree in order to determine the presence of insecticides in *A. floccosus* honeydew. Whitefly colonies were settled on developed leaves and had more than 100 nymphs of different instars. The insecticides flonicamid and pymetrozine were applied onto the foliage at the dose recommended by the producer. Untreated controls were sprayed using only distilled water.

Insecticides were applied until run-off using a wheelbarrow sprayer (Model ATASA MC-25) with a volume of about 5 L per tree.

2.4. Honeydew collection

2.4.1. Under controlled conditions

We collected fresh honeydew from the mealybug *P. citri* daily from 27 April 2017 (+1 day after treatment, DAT) to 2 May 2017 (+5DAT), by placing Parafilm® squares of 5 cm × 5 cm below the infested leaf during 24 h. The collected honeydew for each treatment was labelled and stored at −20 °C in Petri dishes until samples were chemically analysed using HPLC-MS/MS or used in toxicity bioassays (Calvo-Agudo et al., 2019; Hogervorst et al., 2007; Tena et al., 2013b). The number of replicates per treatment, day and tree are provided in Tables 1 and 2.

2.4.2. Under field conditions

Honeydew samples from the mealybug *P. citri* and the whitefly *A. floccosus* were collected on 14 September 2018 (+2DAT). Fresh honeydew was collected over a 24-h period by holding 10 cm wide and 17 cm long plastic punnets below each hemipteran colony. Within the punnets, two pieces of Parafilm® were placed to collect the honeydew. To exclude ants from the samples, we used a wire coated with Tangle-trap (Tangle-foot; Biagro, Valencia, Spain) to hold the punnets. The collected honeydew for each treatment was labelled and stored at −20 °C in Petri dishes until they were used in

Table 1
Insecticide detection and quantification on honeydew excreted by the mealybug *Planococcus citri* feeding on water-treated trees or trees treated with flonicamid between +2 DAT and +5 DAT, under controlled conditions.

Treatment	Tree	Number of samples per tree between +2DAT and +5DAT	Number of samples in which flonicamid was detected	Mean concentration of flonicamid in the tree (ppb)[a]
Control	1	2	0	0
	2	1	0	0
	3	1	0	0
	4	2	0	0
	5	1	0	0
	6	2	0	0
Flonicamid	1	2	1	64.7 ± 64.7
	2	3	3	355.9 ± 110.3
	3	4	3	95.1 ± 50.2
	4	2	1	29.5 ± 29.5
	5	1	1	270
	6	1	0	0

[a] Calculated as the mean ± SE concentration for each tree.

Table 2
Insecticide detection and quantification on honeydew excreted by the mealybug *Planococcus citri* feeding on water-treated trees or trees treated with pymetrozine between +2 DAT and +5 DAT, under controlled conditions.

Treatment	Tree	Number of samples per tree between +2DAT and +5DAT	Number of samples in which pymetrozine was detected	Mean concentration of pymetrozine in the tree (ppb)[a]
Control	1	2	1	28 ± 28
	2	1	0	0
	3	1	0	0
	4	2	0	0
	5	1	0	0
	6	2	0	0
Pymetrozine	1	3	2	10.6 ± 7.1
	2	2	0	0
	3	2	2	33.5 ± 0.5
	4	1	1	6.1
	5	1	1	33
	6	2	2	64.5 ± 29.5
	7	1	0	0
	8	2	0	0
	9	1	1	1.4

[a] Calculated as the average concentration for each tree.

Table 3
Insecticide detection and quantification of honeydew excreted by the mealybug *Planococcus citri* and the whitefly *Aleurothrixus floccosus* feeding on water-treated trees or trees treated with flonicamid under field conditions.

Honeydew producer	Treatment	Tree	Concentration of flonicamid +2DAT (ppb)
Planococcus citri	Control	1	0
		2	0
		3	0
	Flonicamid	1	35.7
		2	24.5
		3	0
Aleurothrixus floccosus	Control	1	0
		2	0
		3	0
		4	0
	Flonicamid	1	0
		2	0
		3	0

Table 4
Insecticide detection and quantification of honeydew excreted by the mealybug *Planococcus citri* and the whitefly *Aleurothrixus floccosus* feeding on water-treated trees or trees treated with pymetrozine under field conditions.

Honeydew producer	Treatment	Tree	Concentration of pymetrozine +2DAT (ppb)
Planococcus citri	Control	1	0
		2	0
		3	0
	Pymetrozine	1	0
		2	20.1
		3	189.8
		4	70.7
Aleurothrixus floccosus	Control	1	0
		2	0
		3	0
		4	9.7
	Pymetrozine	1	27.2
		2	192.2
		3	135.7

the chemical analysis (Hogervorst et al., 2007; Tena et al., 2013b). Honeydew was labelled with information on the corresponding honeydew producer species, treatment, and tree number. The number of replicates per treatment, day and tree are provided in Tables 3 and 4.

2.5. Chemical analysis of honeydew samples

The presence and concentration of flonicamid and pymetrozine in the honeydew samples from both assays were further analysed using HPLC-MS/MS. Under controlled conditions, we collected honeydew samples excreted by the mealybug *P. citri* between +2 DAT and +5DAT. We used nine samples of honeydew excreted by mealybugs feeding on water-treated trees derived from six different trees; twelve samples of honeydew excreted by mealybugs feeding on trees treated with flonicamid derived from six trees; and fifteen samples of honeydew excreted by mealybugs feeding on trees treated with pymetrozine derived from nine trees (replicates per treatment and trees are provided in Tables 1 and 2).

Under field conditions, we collected honeydew samples excreted by the mealybug *P. citri* and the whitefly *A. floccosus* +2DAT. In total, after discarding some samples because of the small amount of honeydew collected, we analysed six samples of honeydew excreted by mealybugs feeding on water-treated trees derived from three trees; four samples of honeydew excreted by mealybugs feeding on trees treated with flonicamid from three trees; and seven samples of honeydew excreted by mealybugs feeding on trees treated with pymetrozine from four trees (replicates per treatment and trees are provided in Tables 3 and 4). For

A. floccosus, we analysed eight samples of honeydew excreted by mealybugs feeding on water-treated trees derived from five trees; six samples of honeydew excreted by mealybugs feeding on trees treated with flonicamid from three trees and six samples of honeydew excreted by mealybugs feeding on trees treated with pymetrozine from three trees (Tables 3 and 4).

The numbers of honeydew droplets excreted by *P. citri* and *A. floccosus* were estimated following the methodology described by Calvo-Agudo et al. (2019).

2.5.1. Insecticide extraction from honeydew

All honeydew droplets from the same honeydew producer species, same tree and day were dissolved in 200 μL of 50% methanol. This diluent solution was deposited on top of the Parafilm® piece containing the honeydew droplets. The solution and the honeydew droplets were stirred gently with the same pipette to dissolve the honeydew and then filtered using acrodisc syringe filters of 13 mm with 0.2 μm PTFE (Pall Corporation, New York, USA). Samples were drawn into 250 μL propylene inserts (Agilent technologies) and subsequently frozen at −20 °C for HPLC-MS/MS analysis.

2.6. Chemical analysis using HPLC-MS/MS

The HPLC-MS/MS analysis was performed by using an infinity Ultra-High-performance Liquid Chromatography 1260 system coupled to Triple Quad Mass Spectrometry 6410 from Agilent Technologies (Santa Clara, CA, USA).

The chromatographic separation was obtained using a Luna®

C18 - 3 μm column (100 Å, 150 × 2.1 mm; Phenomenex, Torrance, CA, USA). The analytical column temperature was kept at 25 °C and the volume injected was 5 μL. The mobile phases were (A) Milli-Q water and (B) methanol, both with a 0.1% of formic acid. Working in isocratic conditions with an 80% of A and a 20% of B. The flow rate was 0.3 mL min^{-1}.

The ionization source was working in positive ionization mode (ESI+) with the following parameters: drying gas (nitrogen) flow of 11 L min^{-1} at 300 °C, nebulizer pressure of 30 psi and capillarity voltage of 4000 V. The Triple Quadrupole HPLC worked in SRM (selected reaction monitoring) mode. The MS/MS transitions were three for pymetrozine and two for flonicamid, as reported in detail in Table S1.

2.6.1. Method validation and quality control

The calibration curve of the MS/MS analysis was performed using external standards dissolved in methanol, a concentration range of 2.5−25 ng mL^{-1} (six points) achieved by weighted least squares linear regression model $(1/x^2)$. Each curve was obtained by two independent injections. The calibration curves have co-efficients of determination $(R^2) > 0.99$. The chromatograms were acquired and processed by Qualitative and Quantitative Mass Hunter Analysis software (Version 10.0) supplied by Agilent Technologies. Figs. S1−S4 show several chromatograms that illustrate the method's performance. The limit of quantification (LOQ) and the limit of detection (LOD) were established as minimum concentrations of the analyte that can be the detected in spiked samples with S/N (signal-to-noise), for the quantifier transition, ≥ 3 for LOD and ≥ 10 for LOQ (with the other transitions visible). LOD values were 0.007 ng g^{-1} for flonicamid, 0.660 ng g^{-1} for pymetrozine and LOQ values were 0.020 ng g^{-1} for flonicamid and 2.000 ng g^{-1} for pymetrozine.

2.7. Mortality of beneficial insects

Anagyrus vladimiri and *S. rueppellii* were fed on honeydew excreted by *P. citri* feeding on trees that had been sprayed three days before with flonicamid, pymetrozine or distilled water (control) under controlled conditions. For *S. rueppellii,* we individually confined newly emerged and unfed adults in 5.3-cm-diameter Petri dishes with 3-cm-diameter holes covered with muslin mesh to allow ventilation. Thirty replicates per treatment were carried out (Calvo-Agudo et al., 2019). For *A. vladimiri*, we used groups of ten newly emerged and unfed females. These females were grouped in 5.3-cm-diameter Petri dishes with 3-cm-diameter holes covered with muslin mesh to allow ventilation. Ten replicates per treatment were carried out (100 individuals per treatment). Parafilm® pieces with honeydew of each treatment were defrosted and observed under the binocular to check for the presence of honeydew. Honeydew was administered *ad libitum* and renewed daily to avoid crystallization (Hogervorst et al., 2007). To ensure that honeydew had been provided *ad libitum,* the presence of honeydew on the Parafilm® removed was checked after the renewal to assess that not all honeydew had been consumed. A piece of wet cotton wool was also placed and renewed daily to provide sufficient moisture. Petri dishes containing the beneficial insects were kept undisturbed in the climatic chambers for 72 h and afterwards mortality was assessed.

2.8. Sublethal effects on beneficial insects

2.8.1. Parasitic wasp longevity

After 72 h, between one and seven surviving females per replicate of the mortality experiment explained above were placed individually into glass vials (subreplicates) of 3 cm high and 0.8 cm diameter covered with wet cotton wool (number of individuals per replicate in Table S2). Parafilm® pieces with honeydew of each treatment were: defrosted, checked for the presence of honeydew; cut into pieces of different sizes (ca. 1.5−3 cm^2 depending on the quantity of honeydew on each piece of Parafilm®) to provide honeydew *ad libitum* (Calvo-Agudo et al., 2019); and placed in the glass vials. Diets were administered daily for each treatment and survival was checked. Glass vials with parasitic wasps were kept in a climate chamber until all individuals had died. Each surviving female was used as replicate because there were no significant differences between replicates (females coming from the same Petri dish) in any treatment. Therefore, we analysed 58 parasitic wasp individuals fed on honeydew from mealybugs feeding on trees treated with distilled water only, 56 on honeydew from mealybugs feeding on trees treated with flonicamid and 52 with pymetrozine.

2.8.2. Parasitism and encapsulation

After 72 h, two or three surviving females per replicate were individually placed in 5.3-cm-diameter Petri dishes (subreplicates) with 3-cm-diameter holes covered with muslin mesh to allow ventilation (number of individuals per replicate in Table S3). Parafilm® pieces with honeydew of each treatment were: defrosted; checked for the presence of honeydew; cut into pieces of different sizes (ca. 1.5−3 cm^2) to provide honeydew *ad libitum* (Calvo-Agudo et al., 2019); and placed in the Petri dishes. Petri dishes also contained a piece of wet cotton wool, one *A. vladimiri* male previously fed on honey to allow mating and five third-instar *P. citri* hosts settled on a green bean. One day later, parasitic wasps were removed and the Petri dishes were kept in the climatic chamber for seven days. Then, the number of mummified (successful parasitism), dead and live mealybugs were counted. Live mealybugs were dissected on a drop of deionized water using entomological needles and scalpels under a stereo microscope to check for encapsulated eggs. We analysed the number of parasitized mealybugs (mummified and alive with encapsulated eggs) and encapsulation for: 27 parasitic wasp individuals fed on honeydew from mealybugs feeding on trees treated with distilled water only, 26 on honeydew from mealybugs feeding on trees treated with flonicamid and 27 with pymetrozine.

2.9. Data analysis

To analyse the mortality of the parasitic wasp and the hoverfly after feeding on honeydew for three days (lethal effect), we used a generalized linear model with quasi-binomial distribution. The mortality of the parasitic wasps was calculated as the number of dead parasitic wasps divided by total number of parasitic wasps per Petri dish. In both analyses, honeydew type was the explanatory variable and mortality the dependent variable. A Bonferroni post-hoc test using the "multcomp" package enabled pairwise comparisons between honeydew treatments.

We used different approaches to analyse sublethal effects of both insecticides present in the honeydew on the parasitic wasp: survivorship, number of parasitized mealybugs and encapsulation rate. The effect of the honeydew treatments on the survival of the parasitic wasp was represented by Kaplan−Meier survivorship curves and analysed by a Cox's Proportional Hazards model using the survival functions of the "survival" package. For this, we first checked that there were no significant differences between replicates (females coming from the same Petri dish used in mortality assays) in any treatment using Cox's Proportional Hazards models [Survivorship of parasitic wBedrockasp females fed on honeydew excreted by mealybugs feeding on trees treated with: water ($\chi^2{}_9 = 10.44$, $P = 0.3$), flonicamid ($\chi^2{}_9 = 14.76$, $P = 0.1$) or

pymetrozine ($\chi^2_7 = 10.1$, $P = 0.3$) (number of individuals per replicate in Table S2)]. Then, we used each female as replicate. Parasitism was calculated by summing the number of successfully parasitized hosts, dead hosts with encapsulated eggs and alive hosts with encapsulated eggs divided by the total number of hosts:

$$Parasitism = \frac{\begin{array}{c} number\ of\ successfully\ parasitized\ hosts+ \\ dead\ hosts\ with\ encapsulated\ eggs+ \\ alive\ hosts\ with\ encapsulated\ eggs \end{array}}{Total\ number\ of\ hosts}$$

Encapsulation was calculated by summing the number of dead hosts with encapsulated eggs and live hosts with encapsulated eggs and divided by the number of parasitized hosts:

$$Encapsulation = \frac{\begin{array}{c} number\ of\ dead\ hosts\ with\ encapsulated\ eggs+ \\ live\ hosts\ with\ encapsulated\ eggs \end{array}}{Number\ of\ parasitized\ hosts}$$

Both sublethal effects were then statistically analysed using a generalized linear mixed model with treatment as explanatory factor and replicate (parasitic wasps from the same Petri dish) as random factor using the "glmer" package. We assumed Poisson and binomial distributions for the number of eggs parasitized and encapsulation rates, respectively. All tests performed were analysed using R (version 3.3.2 for Mackintosh).

3. Results

3.1. Detection and quantification of insecticides under controlled conditions

Under controlled conditions, flonicamid was detected in mealybug-produced honeydew from five out of the six trees treated with this insecticide and in 69.2% of the samples from these six trees (Table 1). These contaminated samples contained 215.8 ± 52.3 ng of flonicamid/mL of honeydew (ppb). No flonicamid was detected in honeydew produced by mealybugs feeding on water-treated trees.

Pymetrozine was detected in mealybug-produced honeydew from six out of the nine trees treated with this insecticide and in 60% of the samples from these nine trees (Table 2). These contaminated samples contained 37 ± 12.1 ng of pymetrozine/mL of honeydew (ppb). Pymetrozine was detected in one sample out of the nine samples analysed from the six control trees at a concentration of 56 ng of pymetrozine/mL of honeydew (ppb).

3.2. Detection and quantification of insecticides in honeydew excreted by two species of honeydew producers under field conditions

Two days after insecticide application in the field, flonicamid was detected in mealybug-produced honeydew from two out of the three trees treated (Table 3). These contaminated samples contained 30.1 ± 5.6 ng of flonicamid/mL of honeydew (ppb). In contrast, no flonicamid was detected in honeydew excreted by the whitefly *A. floccosus*.

Two days after insecticide application in the field, pymetrozine was detected in mealybug-produced honeydew from three out of the four trees treated (Table 4). These contaminated samples contained 93.6 ± 50.3 ng of pymetrozine/mL of honeydew (ppb). Pymetrozine was also detected in whitefly-produced honeydew from all trees treated with this insecticide (Table 4). These contaminated samples contained 118.4 ± 48.4 ng of pymetrozine/mL of honeydew (ppb). Pymetrozine was detected in one tree out of

the four control trees at a concentration of 9.7 ng of pymetrozine/mL of honeydew (ppb).

3.3. Mortality of beneficial insects

All *S. rueppellii* hoverflies survived after three days feeding on honeydew excreted by mealybugs feeding on water-treated trees. In contrast, $56 \pm 10\%$ of the hoverflies died in the flonicamid treatment and $22.2 \pm 8\%$ in the pymetrozine treatment. Mortality significantly differed among the three treatments (GLM based on binomial distribution, $F_{2, 53} = 72.17$, $P < 0.015$) (Fig. 1a).

Mortality of the parasitic wasp *A. vladimiri* was similar when it fed on honeydew excreted by mealybugs feeding on water-treated trees ($6.1 \pm 2.7\%$), trees treated with flonicamid ($11 \pm 4.8\%$) or pymetrozine ($14 \pm 3.4\%$) (GLM based on quasi-binomial distribution, $F_{2, 27} = 1.21$, $P = 0.31$) (Fig. 1b).
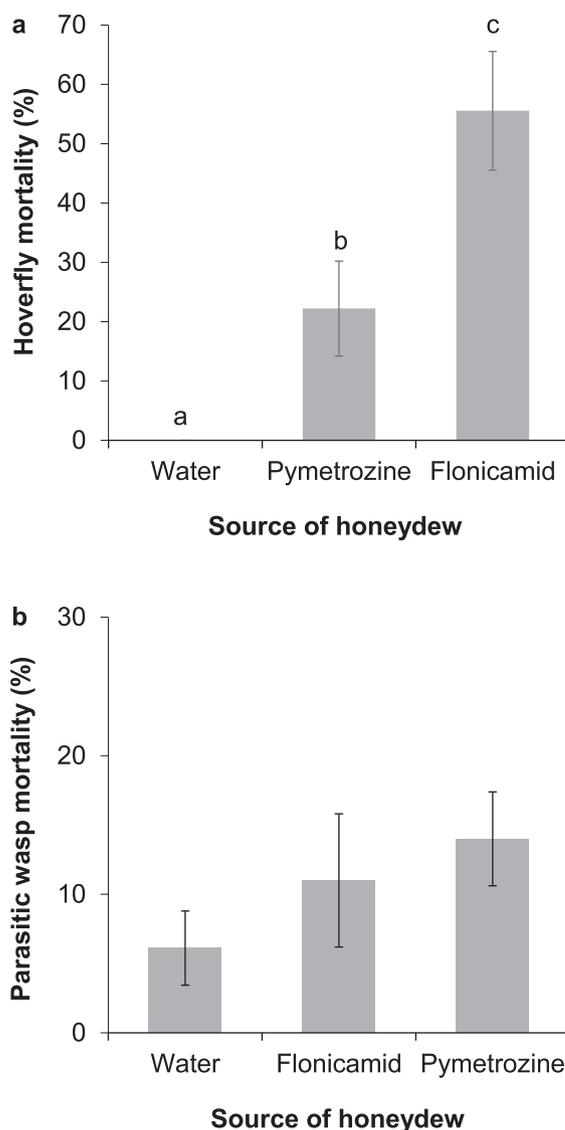

Fig. 1. Mortality (mean $\pm$ SE) of a) the parasitic wasp *Anagyrus vladimiri* (N = 10 replicates of 10 females each per treatment) and b) the hoverfly *Sphaerophoria rueppellii* (N = 30 replicates per treatment) fed on honeydew of *Planococcus citri* feeding on water-treated trees or on honeydew of *P. citri* feeding on trees treated with the insecticides flonicamid or pymetrozine. Mortality was assessed after feeding on honeydew for 72 h. Columns with different letters are significantly different from each other (GLM with quasibinomial distribution followed by a Bonferroni test, $P < 0.05$).
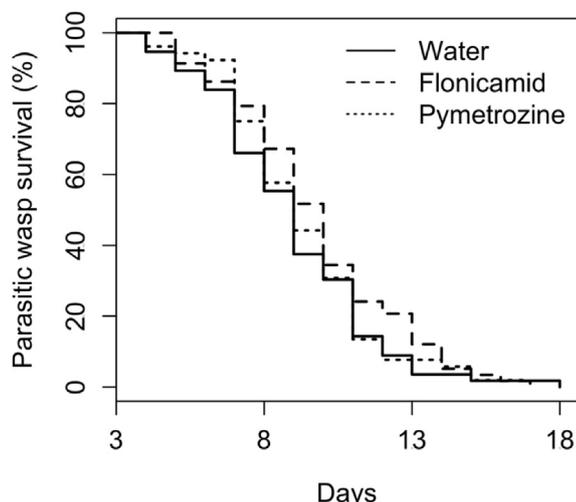
Fig. 2. Survival curve (mean ± SE) estimated by Kaplan-Meier of the parasitic wasp *Anagyrus vladimiri*. ($N_{Honeydew}$ = 58, $N_{Honeydew\ with\ flonicamid}$ = 56, $N_{Honeydew\ with\ pymetrozine}$ = 52), fed on honeydew of *Planococcus citri* feeding on water-treated trees or on honeydew of *P. citri* feeding on trees treated with the insecticides flonicamid or pymetrozine.

3.4. Sublethal effects on parasitic wasps

The longevity of the surviving parasitic wasps was similar when feeding on honeydew excreted by mealybugs feeding on water-treated trees (9.7 ± 0.4 days), trees treated with flonicamid (8.7 ± 0.4) or pymetrozine (9 ± 0.4) (Cox's Proportional Hazards: $\chi^2_2 = 1.97$, $P = 0.37$) (Fig. 2).

After feeding on honeydew for three days, parasitic wasps that fed on honeydew excreted by mealybugs feeding on trees treated with distilled water parasitized the same number of hosts (3.17 ± 0.27 parasitized mealybugs) as those fed on honeydew excreted by mealybugs feeding on trees treated with flonicamid (2.98 ± 0.18 parasitized mealybugs) or pymetrozine (3.47 ± 0.25 parasitized mealybugs) (GLMM based on Poisson, $\chi^2_2 = 1.25$, $P = 0.54$). Among the parasitized hosts, the percentage of encapsulated eggs was similar for the three treatments (water: 52.6 ± 5.1%; flonicamid: 52.8 ± 10.1%; pymetrozine: 55.7 ± 3.9%; GLMM based on binomial, $\chi^2_2 = 0.04$, $P = 0.98$).

4. Discussion

Our results demonstrate that IPM-recommended insecticides, such as flonicamid and pymetrozine, reach honeydew at concentrations that can be toxic to beneficial insects. These insecticides were selected because they are phloem-transported and are applied in many crops including fruit trees, cereals, potatoes or vegetables to control numerous pests such as whiteflies, aphids, planthoppers or leafhoppers (Belchim, 2020; Syngenta, 2020). Flonicamid and pymetrozine are commonly foliar- or soil-applied. Here, we selected and tested the foliar application of both insecticides because it is the most common mode of application in citrus crops, as well as in other crops (Colomer et al., 2011; Qureshi et al., 2014). When sprayed, insecticides might reach honeydew through three different pathways: i) by contact with the honeydew producer - insecticides can be directly absorbed by the body of honeydew producers while it is sprayed, and honeydew producers excrete the insecticide via their honeydew; ii) through the plant - after spraying, systemic insecticides are translocated to all parts of the plant, the honeydew producer feeds on the plant and excretes the insecticide via its honeydew, and iii) insecticides can directly contaminate honeydew already present in crop. The third pathway can be excluded in our study because we did not collect honeydew just after the application. The collection started 48 h after the insecticide application and the honeydew collected was excreted during the next 24 h. The other two contamination routes cannot be differentiated and might occur simultaneously because both insecticides can act by contact and ingestion (Belchim, 2020; Syngenta, 2020).

Flonicamid and pymetrozine were detected in ca. 60−70% of the honeydew samples collected from the mealybug *P. citri* under controlled and field conditions. These results demonstrated that these insecticides are excreted by the mealybug under different conditions and at different times after their applications. In the field, where we collected honeydew excreted by the mealybug *P. citri* and the whitefly *A. floccosus,* flonicamid was detected in samples of honeydew excreted by the mealybug but not by the whitefly. This difference between hemipteran species might be explained by the different feeding behavior of honeydew producers and the physiochemical properties of flonicamid. Whiteflies such as *A. floccosus* feed mostly on plant phloem and stylets occasionally penetrate the xylem (Lei et al., 1997), whereas mealybugs such as *P. citri* feed frequently on both phloem and xylem (Obok et al., 2018). Therefore, *A. floccosus* is, compared to *P. citri,* less likely to excrete insecticides that move through the xylem. At 20 °C, flonicamid can move through xylem but, contrary to pymetrozine, does not have optimal phloem mobility and is less retained in the phloem sieve tubes (University of Herthfordshire, 2020; Bromilow et al., 1990). Overall, these results show that the presence and toxicity of insecticides via honeydew can vary not only among insecticides but also among honeydew-producing species. Therefore, further research that evaluates the presence of insecticides in honeydew should also take the honeydew-producing species into consideration.

Among honeydew-producing species, those that are tolerant or resistant to insecticides might excrete honeydew for a longer period of time. In our experiments, we used *P. citri* because it is tolerant to the insecticides pymetrozine and flonicamid (El-Zahi et al., 2016; Rezk et al., 2019). Since this mealybug is tolerant to these insecticides, it might excrete contaminated honeydew from a few days after the treatment, as occurred in our experiments, until these insecticides or their metabolites are completely degraded in the plant. The metabolites of flonicamid and pymetrozine can remain in citrus for more than 60 and 21 days after their application (Belchim, 2020; Syngenta, 2020). There are many other honeydew-producing species that are tolerant or resistant to insecticides. For example, the silverleaf whitefly *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) and the green peach aphid *Myzus persicae* (Sulzer) (Hemiptera: Aphididae) feed on hundreds of plant species of more than forty families (Brown et al., 1995; Holman, 2009) and have developed resistance to more than 40 and 70 active ingredients, respectively (Van Leeuwen et al., 2010), including pymetrozine (Gorman et al., 2010; Qiong et al., 2012). Therefore, the route of exposure described here, where tolerant/resistant hemipterans excrete contaminated honeydew, can be common in numerous crops.

The mortality of the hoverfly *S. rueppellii* was higher when it fed on honeydew contaminated by flonicamid than pymetrozine. However, honeydew contaminated with these insecticides was harmless to the parasitic wasp *A. vladimiri*. The hoverfly was also more susceptible than the parasitic wasp in our previous study with neonicotinoids (Calvo-Agudo et al., 2019). This difference between the two beneficial insects is likely because hoverflies are more sensitive to insecticides than parasitoids (Calvo-Agudo et al., 2019; Sanchez-Bayo, 2014), have a greater feeding rate (Cresswell et al., 2014), and/or a lower detoxification capacity (Manjon et al.,

2018). Some studies have demonstrated lethal effect of flonicamid and pymetrozine, as well as a range of sublethal effects including a change in the feeding behavior, developmental period of nymphs, adult longevity, and fecundity of beneficial insects when these had been in contact with the insecticide residue (Jansen et al., 2011; Joseph et al., 2011; Moens et al., 2011). For instance, flonicamid increases the mortality of the parasitic wasp *Aphidius rhopalosiphi* (Hymenoptera: Braconidae) (Jansen et al., 2011) and affects the reproductive performance (egg hatching and viable eggs per female) of the hoverfly *Episyrphus balteatus* (Diptera: Syrphidae) (Moens et al., 2011). Similarly, several studies have reported lethal and sublethal effects of pymetrozine. The parasitic wasp *A. rhopalosiphi* tends to die after contacting treated glass plates during 48 h (Jansen et al., 2011) and the mortality of immature individuals of the hoverfly *E. balteatus* was also affected (Jansen et al., 2011). Sublethal effects include effects on *Aphidius ervi* (Hymenoptera: Braconidae) pre-imaginal development inside contaminated hosts (Joseph et al., 2011); male-biased sex ratio in *A. ervi* (Joseph et al., 2011); reduced host feeding in *Neochrysocharis formosa* (Hymenoptera: Eulophidae) (Tran et al., 2004); lower predation rate in *Tenuisvalvae notata* (Coleoptera: Coccinellidae) larvae (Barbosa et al., 2018); or inability to discriminate between contaminated or uncontaminated hosts (Joseph et al., 2011). Most studies for both insecticides, however, did only consider toxicity through direct application or contact with residues. Only few studies took into account oral exposure through contaminated prey for predators (Colomer et al., 2011) or contaminated hosts for immature parasitoids (Joseph et al., 2011), but none explored the potential toxicity of contaminated carbohydrate sources such as floral and extrafloral nectar and honeydew. Therefore, to the best of our knowledge, this is the first study that considers oral toxicity of these insecticides in a carbohydrate source, although both insecticides are present not only in honeydew (presented here) but also in nectar and pollen (Azpiazu, 2020; Kyriakopoulou et al., 2017). Further studies should evaluate potential sublethal effects of pymetrozine and flonicamid on hoverflies when they feed on contaminated honeydew. In our study, flonicamid was more toxic than pymetrozine but these results are based on the lethal effects of these insecticides on *S. rueppellii*. As explained above, both insecticides can cause other detrimental effects that should be explored to evaluate the toxicity of these insecticides when hoverflies feed on carbohydrate sources contaminated with insecticides.

5. Conclusion

This study demonstrates that IPM-recommended insecticides such as pymetrozine and flonicamid may be present in honeydew excreted by hemipterans that are feeding on treated trees. We also show, for the first time, that the presence of insecticides in hemipteran honeydew depends on the hemipteran species. The results presented here, together with those of Calvo-Agudo et al. (2019) and Quesada et al. (2020) indicate that honeydew contaminated with insecticides can occur in many different agroecosystems. This route of exposure has been demonstrated for three species of honeydew producers belonging to three different families, five systemic insecticides with four different modes of action and translocation routes, and two plant species. Moreover, our results also suggest that honeydew-producing species that are tolerant or resistant to insecticides might excrete contaminated honeydew for longer periods. Therefore, contaminated honeydew is likely to affect a much wider range of beneficial insects than contaminated nectar and, thus, should be included in future environmental risk assessments.

Data accessibility

Raw data can be accessed from the Dryad data repository: https://datadryad.org/stash/share/T6DLcRPXtEBY2GwfZdLHxYg3johK-xJS3GGvJhdDRdM.

Supporting data are accessible in the electronic supplementary material: Table S1, Table S2, Table S3 and Fig. S1, Fig. S2, Fig. S3, Fig. S4.

Author contributions

Miguel Calvo-Agudo: Conceptualization, Methodology, Formal analysis, Investigation, Writing-Original draft, Visualization; Joel González-Cabrera: Conceptualization, Writing-Reviewing and Editing; Daniele Sadutto: Methodology, Formal analysis; Yolanda Picó: Methodology, Writing-Reviewing and Editing; Alberto Urbaneja: Writing-Reviewing and Editing; Marcel Dicke: Writing-Reviewing, Supervision and Editing; Alejandro Tena: Conceptualization, methodology, formal analysis, Writing-Reviewing and Editing, Visualization, Supervision and Funding Acquisition.

Funding

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.

Acknowledgements

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2020.115581.

References

Azpiazu, C., 2020. Evaluation of Flowering Plant Strips and the Risk of Pesticides on Pollinators in Melon Agro-Ecosystems. PhD Thesis at Universidad Politécnica de Madrid.

Barbosa, P.R.R., Oliveira, M.D., Barros, E.M., Michaud, J.P., Torres, J.B., 2018. Differential impacts of six insecticides on a mealybug and its coccinellid predator. Ecotoxicol. Environ. Saf. 147, 963–971. https://doi.org/10.1016/j.ecoenv.2017.09.021.

Belchim (Teppeki; Flonicamid). https://belchim.es/productos/teppeki/ (Accessed 20 July 2020).

Bonmatin, J.M., Giorio, C., Girolami, V., Goulson, D., Kreutzweiser, D.P., Krupke, C., Liess, M., Long, E., Marzaro, M., Mitchell, E.A., Noome, D.A., Simon-Delso, N., Tapparo, A., 2015. Environmental fate and exposure; neonicotinoids and fipronil. Environ. Sci. Pollut. Res. 22, 35–67. https://doi.org/10.1007/s11356-014-3332-7.

Bromilow, R.H., Chamberlain, K., Evans, A.A., 1990. Physicochemical aspects of phloem translocation of herbicides. Weed Sci. Soc. Am. 38, 305–314.

Brown, J.K., Forhlich, D.R., Rosell, R.C., 1995. The sweetpotato or silverleaf whiteflies: biotypes of *Bemisia tabaci* or a species complex? Annu. Rev. Entomol. 40, 511–534. https://doi.org/10.1146/annurev.ento.40.1.511.

Calabuig, A., Tena, A., Wäckers, F.L., Fernández-Arrojo, L., Plou, F.J., Garcia-Marí, F., Pekas, A., 2015. Ants impact the energy reserves of natural enemies through the shared honeydew exploitation. Ecol. Entomol. 40, 687–695. https://doi.org/10.1111/een.12237.

Calvo-Agudo, M., González-Cabrera, J., Picó, Y., Calatayud-Vernich, P., Urbaneja, A., 2019. Neonicotinoids in excretion product of phloem-feeding insects kill beneficial insects. Proc. Natl. Acad. Sci. Unit. States Am. 116, 16817–16822. https://doi.org/10.1073/pnas.1904298116.

Cameron, S.A., Corbet, S.A., Whitfield, J.B., 2019. Bumble bees (Hymenoptera: Apidae: *Bombus terrestris*) collecting honeydew from the giant willow aphid (Hemiptera: Aphididae). J. Hymenoptera Res. 68, 75–83. https://doi.org/10.3897/jhr.68.30495.

Colomer, I., Aguado, P., Medina, P., Heredia, R.M., Fereres, A., Belda, J.E., Viñuela, E., 2011. Field trial measuring the compatibility of methoxyfenozide and flonicamid with *Orius laevigatus* Fieber (Hemiptera: Anthocoridae) and *Amblyseius swirskii* (Athias-Henriot) (Acari: Phytoseiidae) in a commercial pepper greenhouse. Pest Manag. Sci. 67, 1237–1244. https://doi.org/10.1002/ps.2173.

Cresswell, J.E., Robert, F.X.L., Florance, H., Smirnoff, N., 2014. Clearance of ingested neonicotinoid pesticide (imidacloprid) in honey bees (*Apis mellifera*) and bumblebees (*Bombus terrestris*). Pest Manag. Sci. 70, 332–337.

Desneux, N., Decourtye, A., Delpuech, J.-M., 2007. The sublethal effects of pesticides on beneficial arthropods. Annu. Rev. Entomol. 52, 81–106. https://doi.org/10.1146/annurev.ento.52.110405.091440.

Downes, W.L., Dahlem, G.A., 1987. Keys to the evolution of Diptera: role of Homoptera. Environ. Entomol. 16, 847–854. https://doi.org/10.1093/ee/16.4.847.

El-Zahi, E.Z.S., El-Salam Aref, S.A., Mohammad Korish, S.K., 2016. The cotton mealybug, *Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae) as a new menace to cotton in Egypt and its chemical control. J. Plant Protect. Res. 56, 111–115. https://doi.org/10.1515/jppr-2016-0017.

European Food Safety Authority, 2018. Evaluation of the data on clothianidin, imidacloprid and thiamethoxam for the updated risk assessment to bees for seed treatments and granules in the EU. EFSA Support. Publ. 15 https://doi.org/10.2903/sp.efsa.2018.EN-1378.

Gorman, K., Slater, R., Blande, J.D., Clarke, A., Wren, J., McCaffery, A., Denholm, I., 2010. Cross-resistance relationships between neonicotinoids and pymetrozine in *Bemisia tabaci* (Hemiptera: Aleyrodidae). Pest Manag. Sci. 66, 1186–1190. https://doi.org/10.1002/ps.1989.

Hagen, K.S., 1962. Biology and ecology of predaceous Coccinellidae. Annu. Rev. Gemmolil. 7, 289–326. https://doi.org/10.1146/annurev.en.07.010162.001445.

Heimpel, G.E., Jervis, M., 2005. Does floral nectar improve biological control by parasitoids? In: Wackers, F.L., van Rijn, P.C.J., Bruin, J. (Eds.), Plant-Provided Food for Carnivorous Insects: A Protective Mutualism and its Applications. Cambridge University Press, Cambridge, pp. 267–304. https://doi.org/10.1017/CBO9780511542220.010. Cambridge University Press, Cambridge, UK.

Heimpel, G.E., Mills, N.J., 2017. Biological Control: Ecology and Applications. Cambridge University Press.

Henry, M., Béguin, M., Requier, F., Rollin, O., Odoux, J.F., Aupinel, P., Aptel, J., Tchamitchian, S., Decourtye, A., 2012. A common pesticide decreases foraging success and survival in honey bees. Science 336, 348–350. https://doi.org/10.1126/science.1215039.

Herren, H.R., Neuenschwander, P., 1991. Biological control of cassava pests in Africa. Annu. Rev. Entomol. 36, 257–283. https://doi.org/10.1146/annurev.en.36.010191.001353.

Hogervorst, P.A.M., Wäckers, F.L., Romeis, J., 2007. Detecting nutritional state and food source use in field-collected insects that synthesize honeydew oligosaccharides. Funct. Ecol. 21, 936–946. https://doi.org/10.1111/j.1365-2435.2007.01297.x.

Hölldobler, B., Wilson, E.O., 1990. The Ants. Harvard University Press. https://doi.org/10.1002/ejoc.201200111.

Holman, J., 2009. Host Plant Catalog of Aphids. Springer Science & Business Media. https://doi.org/10.1007/978-1-4020-8286-3.

Insecticide Resistance Action Committee, 2020. Modes of Action (MoA) Classification | IRAC. https://www.irac-online.org/modes-of-action/. (Accessed 20 July 2020).

Jansen, J.P., Defrance, T., Warnier, A.M., 2011. Side effects of flonicamide and pymetrozine on five aphid natural enemy species. BioControl 56, 759–770. https://doi.org/10.1007/s10526-011-9342-1.

Joseph, J.R., Ameline, A., Couty, A., 2011. Effects on the aphid parasitoid *Aphidius ervi* of an insecticide (Plenum®, pymetrozine) specific to plant-sucking insects. Phytoparasitica 39, 35–41. https://doi.org/10.1007/s12600-010-0134-4.

Kampfraath, A.A., Giesen, D., van Gestel, C.A.M., Le Lann, C., 2017. Pesticide stress on plants negatively affects parasitoid fitness through a bypass of their phytophage hosts. Ecotoxicology 26, 383–395. https://doi.org/10.1007/s10646-017-1771-x.

Konrad, R., Wäckers, F.L., Romeis, J., Babendreier, D., 2009. Honeydew feeding in the solitary bee *Osmia bicornis* as affected by aphid species and nectar availability. J. Insect Physiol. 55, 1158–1166. https://doi.org/10.1016/j.jinsphys.2009.08.012.

Kyriakopoulou, K., Kandris, I., Pachiti, I., Kasiotis, K.M., Spyropoulou, A., Santourian, A., Kitromilidou, S., Pappa, G., Glossioti, M., 2017. Collection and analysis of pesticide residue data for pollen and nectar – final Report. EFSA Support. Publ. 14 https://doi.org/10.2903/sp.efsa.2017.en-1303.

Lee, J.C., Andow, D.A., Heimpel, G.E., 2006. Influence of floral resources on sugar feeding and nutrient dynamics of a parasitoid in the field. Ecol. Entomol. 31, 470–480. https://doi.org/10.1111/j.1365-2311.2006.00800.x.

Lei, H., Tjallingii, W.F., Lenteren, J.C., 1997. Effect of tethering during EPG recorded probing by adults of the greenhouse whitefly. J. Appl. Entomol. 121, 211–217. https://doi.org/10.1111/j.1439-0418.1997.tb01395.x.

Losey, J.E., Vaughan, M., 2006. The economic value of ecological services provided by insects. Bioscience 56, 311–323.

Lundgren, J.G., 2009. Relationships of Natural Enemies and Non-prey Foods. Springer Science & Business Media. https://doi.org/10.1007/978-1-4020-9235-0.

Manjon, C., Troczka, B.J., Zaworra, M., Beadle, K., Randall, E., Hertlein, G., Singh, K.S., Zimmer, C.T., Homem, R.A., Lueke, B., Reid, R., Kor, L., Kohler, M., Benting, J., Williamson, M.S., Davies, T.G.E., Field, L.M., Bass, C., Nauen, R., 2018. Unravelling the molecular determinants of bee sensitivity to neonicotinoid insecticides. Curr. Biol. 28, 1137–1143. https://doi.org/10.1016/j.cub.2018.02.045 e5.

Moens, J., de Clercq, P., Tirry, L., 2011. Side effects of pesticides on the larvae of the hoverfly *Episyrphus balteatus* in the laboratory. Phytoparasitica 39, 1–9. https://doi.org/10.1007/s12600-010-0127-3.

Obok, E., Wetten, A., Allainguillaume, J., 2018. Electropenetrography application and molecular-based virus detection in mealybug (Hemiptera: Pseudococcidae) vectors of Cacao swollen shoot virus on *Theobroma cacao* L. Ann. Agric. Sci. 63, 55–65. https://doi.org/10.1016/j.aoas.2018.04.004.

Planes, L., Catalan, J., Tena, A., Porcuna, J.L., Jacas, J.A., Izquierdo, J., Urbaneja, A., 2013. Lethal and sublethal effects of spirotetramat on the mealybug destroyer, *Cryptolaemus montrouzieri*. J. Pest. Sci. 86 (2004), 321–327. https://doi.org/10.1007/s10340-012-0440-3.

Powney, G.D., Carvell, C., Edwards, M., Morris, R.K.A., Roy, H.E., Woodcock, B.A., Isaac, N.J.B., 2019. Widespread losses of pollinating insects in Britain. Nat. Commun. 10 https://doi.org/10.1038/s41467-019-08974-9.

Qiong, R., Yong-Hua, X.U., Chen, L., Zhang, H.-Y., Jones, C.M., Devine, G.J., Gorman, K., Denholm, I., 2012. Characterisation of neonicotinoid and pymetrozine resistance in strains of *Bemisia tabaci* (Hemiptera: Aleyrodidae) from China. J. Integr. Agric. 11, 321–326. https://doi.org/10.1016/S2095-3119(12)60016-1.

Quesada, C.R., Scharf, M.E., Sadof, C.S., 2020. Excretion of non-metabolized insecticides in honeydew of striped pine scale. Chemosphere 249, 126167. https://doi.org/10.1016/j.chemosphere.2020.126167.

Qureshi, J.A., Kostyk, B.C., Stansly, P.A., 2014. Insecticidal suppression of asian citrus psyllid *Diaphorina citri* (Hemiptera: Liviidae) vector of huanglongbing pathogens. PloS One 9, e112331. https://doi.org/10.1371/journal.pone.0112331.

Rader, R., Bartomeus, I., Garibaldi, L.A., Garratt, M.P.D., Howlett, B.G., Winfree, R., Cunningham, S.A., Mayfield, M.M., Arthur, A.D., Andersson, G.K.S., Bommarc, R., Brittain, C., Carvalheiro, L.G., Chacoff, N.P., Entling, M.H., Foully, B., Freitas, B.M., Gemmill-Herren, B., Ghazoul, J., Griffin, S.R., Gross, C.L., Herbertsson, L., Herzog, F., Hipólito, J., Jaggar, S., Jauker, F., Klein, A.-M., Kleijn, D., Krishnan, S., Lemos, C.Q., Lindström, S.A.M., Mandelik, Y., Monteiro, V.M., Nelson, W., Nilssonl, L., Pattemore, D.E., Pereira, N. de O., Pisanty, G., Potts, S.G., Reemer, M., Rundlöf, M., Sheffield, C.S., Scheper, J., Schüepp, C., Smith, H.C., Stanley, D.A., Stout, J.C., Szentgyörgy, H., Taki, H., Vergara, C.H., Viana, B.F., Woyciechowski, M., 2015. Non-bee insects are important contributors to global crop pollination. Proc. Natl. Acad. Sci. Unit. States Am. 113, 146–151. https://doi.org/10.1073/pnas.1517092112.

Rezk, M., Hassan, A.N.T., El-Deeb, M.F., Shaarawy, N., Dewer, Y., 2019. The impact of insecticides on the cotton mealybug, *Phenacoccus solenopsis* (Tinsley): efficacy on potato, a new record of host plant in Egypt. J. Plant Protect. Res. 59, 50–59. https://doi.org/10.24425/jppr.2019.126042.

Sanchez-Bayo, F., 2014. The trouble with neonicotinoids. Science 346, 806–807. https://doi.org/10.1126/science.1259159.

Sánchez-Bayo, F., Belzunces, L., Bonmatin, J.M., 2017. Lethal and sublethal effects, and incomplete clearance of ingested imidacloprid in honey bees (*Apis mellifera*). Ecotoxicology 26, 1199–1206. https://doi.org/10.1007/s10646-017-1845-9.

Stapel, J.O., Cortesero, A.M., Lewis, W.J., 2000. Disruptive sublethal effects of insecticides on biological control: altered foraging ability and life span of a parasitoid after feeding on extrafloral nectar of cotton treated with systemic insecticides. Biol. Contr. 17, 243–249. https://doi.org/10.1006/bcon.

Syngenta (Plenum; Pymetrozine). https://www.syngenta.com.mx/product/crop-protection/insecticida/plenumr-50-gs (Accessed 20 July 2020).

Tappert, L, Pokorny, T., Hofferberth, J., Ruther, J., 2017. Sublethal doses of imidacloprid disrupt sexual communication and host finding in a parasitoid wasp. Sci. Rep. 7, 1–9. https://doi.org/10.1038/srep42756.

Tena, A., Llácer, E., Urbaneja, A., 2013a. Biological control of a non-honeydew producer mediated by a distinct hierarchy of honeydew quality. Biol. Contr. 67, 117–122. https://doi.org/10.1016/j.biocontrol.2013.07.018.

Tena, A., Pekas, A., Wäckers, F.L., Urbaneja, A., 2013b. Energy reserves of parasitoids depend on honeydew from non-hosts. Ecol. Entomol. 38, 278–289. https://doi.org/10.1111/een.12018.

Tena, A., Wäckers, F.L., Heimpel, G.E., Urbaneja, A., Pekas, A., 2016. Parasitoid nutritional ecology in a community context: the importance of honeydew and implications for biological control. Curr. Opin. Insect Sci. 14, 100–104. https://doi.org/10.1016/j.cois.2016.02.008.

Tran, D.H., Takagi, M., Takasu, K., 2004. Effects of selective insecticides on host searching and oviposition behavior of *Neochrysocharis formosa* (Westwood) (Hymenoptera: Eulophidae), a larval parasitoid of the American serpentine leafminer. Appl. Entomol. Zool. 39, 435–441. https://doi.org/10.1303/aez.2004.435.

University of Herthfordshire, 2020. Pesticide Properties DataBase | PPDB. See. https://sitem.herts.ac.uk/aeru/footprint/es/Reports/562.htm#trans. (Accessed 20 July 2020).

Urbaneja, A., Grout, T.G., Gravena, S., Wu, F., Cen, Y., Stansly, P.A., 2020. Citrus Pests in a Global World. The Genus Citrus. Elsevier Inc. https://doi.org/10.1016/B978-0-12-812163-4.00016-4.

Van Leeuwen, T., Vontas, J., Tsagkarakou, A., Dermauw, W., Tirry, L., 2010. Acaricide resistance mechanisms in the two-spotted spider mite *Tetranychus urticae* and other important Acari: a review. Insect Biochem. Mol. Biol. 40, 563–572. https://doi.org/10.1016/j.ibmb.2010.05.008.

Wäckers, F.L., van Rijn, P.C.J., Bruin, J., 2005. Suitability of (extra-) floral nectar, pollen and honeydew as insect food sources. In: Plant-Provided Food for Carnivorous Insects: A Protective Mutualism and its Applications, pp. 17–74. https://doi.org/10.1017/CBO9780511542220.003.

Wäckers, F.L., van Rijn, P.C.J., Heimpel, G.E., 2008. Honeydew as a food source for natural enemies: making the best of a bad meal? Biol. Contr. 45, 176–184. https://doi.org/10.1016/j.biocontrol.2008.01.007.

Whitehorn, P.R., O'Connor, S., Wackers, F.L., Goulson, D., 2012. Neonicotinoid pesticide reduces bumble bee colony growth and queen production. Science 336, 351–352. https://doi.org/10.1126/science.1215025.