



# Neonicotinoids from coated seeds toxic for honeydew-feeding biological control agents<sup>☆</sup>

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## ARTICLE INFO

### Keywords:

Thiamethoxam  
Parasitic wasp  
Predatory midge  
Soybean aphid  
Seed coating

## ABSTRACT

Seed coating ('seed treatment') is the leading delivery method of neonicotinoid insecticides in major crops such as soybean, wheat, cotton and maize. However, this prophylactic use of neonicotinoids is widely discussed from the standpoint of environmental costs. Growing soybean plants from neonicotinoid-coated seeds in field, we demonstrate that soybean aphids (*Aphis glycines*) survived the treatment, and excreted honeydew containing neonicotinoids. Biochemical analyses demonstrated that honeydew excreted by the soybean aphid contained substantial concentrations of neonicotinoids even one month after sowing of the crop. Consuming this honeydew reduced the longevity of two biological control agents of the soybean aphid, the predatory midge *Aphidoletes aphidimyza* and the parasitic wasp *Aphelinus certus*. These results have important environmental and economic implications because honeydew is the main carbohydrate source for many beneficial insects in agricultural landscapes.

## 1. Introduction

Seed coating ('seed treatment') is the leading delivery method of neonicotinoid insecticides (Frank and Tooker, 2020; Matsuda et al., 2020). Seeds coated with neonicotinoids have been routinely used for major crops such as soybean, wheat, cotton and maize. For example, the seeds of over 50% of soybeans, 52–77% of cotton, and 79–100% of maize sown in the United States were coated with neonicotinoids in 2011 (Douglas and Tooker, 2015; Hurley and Mitchell, 2017), although they increased yield in less than 5% of the cases (Labrie et al., 2020). Seeds coated with neonicotinoids have been extensively used over the last decades because they can be applied against a broad spectrum of insect pests at the beginning of the cropping cycle without the economic costs of spraying (Matsuda et al., 2020). However, evidence is growing for a causal link between the use of neonicotinoids and decreases in biomass and biodiversity of beneficial insects, including pollinators and biological control agents that regulate agricultural pests (Goulson, 2013;

Krupke and Tooker, 2020; Woodcock et al., 2017). Neonicotinoids from coated seeds contaminate floral and extrafloral nectar because these pesticides are taken up systemically by the growing plant and distributed to all tissues (Goulson, 2013; Rundlöf et al., 2015; Whitehorn et al., 2012). Many beneficial insects therefore become exposed to neonicotinoids when they feed on contaminated nectar and pollen (Krischik et al., 2007; Rundlöf et al., 2015).

A recent study under controlled conditions identified a route of exposure of neonicotinoids to beneficial insects that can be more influential than nectar in extensive monocultures (Calvo-Agudo et al., 2019). In these landscapes, honeydew is often the main carbohydrate source for biological control agents because nectar is limited to the brief flowering period of the crop or to the presence of wild flowers or weeds growing in field margins (Lundgren, 2009; Tena et al., 2016). Honeydew is a nutritious and ubiquitous carbohydrate source excreted by hemipteran phloem-feeding insects such as aphids, whiteflies, mealybugs, coccids, and psyllids that feed on crops. Under controlled conditions, plants

<sup>☆</sup> This paper has been recommended for acceptance by Dr Jiayin Dai.

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<https://doi.org/10.1016/j.envpol.2021.117813>

Received 21 May 2021; Received in revised form 5 July 2021; Accepted 18 July 2021

Available online 19 July 2021

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sprayed or watered with systemic insecticides including neonicotinoids can support hemipterans that survive the treatment and excrete contaminated honeydew that is toxic for biological control agents and pollinators (Calvo-Agudo et al., 2019). However, it remains unexplored whether this route of exposure is present in crops grown from neonicotinoid-coated seeds, which represents the main use of neonicotinoids worldwide, and thus has the potential to affect biological control agents at a large scale worldwide<sup>1</sup>. In the United States alone, the ecosystem services provided by biological control agents have been conservatively estimated to be 4.5 billion dollars per year (Losey and Vaughan, 2006).

Soybeans represent a major crop in the United States, with more than 35 million hectares planted in 2018 (Food and Agriculture Organization of the United Nations, 2021), and an estimated 85% or more of it is sown with seeds coated with neonicotinoids combined with fungicides (Hurley and Mitchell, 2017). Neonicotinoid seed treatments are often combined with fungicides, which can synergize the toxic effect of the neonicotinoids in nectar on beneficial insects (Sgolastra et al., 2017). The main target pest of soybean grown from insecticide-coated seeds in the North-Central United States and Eastern Canada is the soybean aphid, *Aphis glycines*, an invasive pest first documented in North America in 2000 (Ragsdale et al., 2011). However, the usefulness of these seed treatments in controlling soybean aphid has been questioned (Krupke et al., 2017; Mourtzinis et al., 2019). The protection period of coated seeds lasts approximately 3–4 weeks after planting (Krupke et al., 2017; Mourtzinis et al., 2019; Seagraves and Lundgren, 2012), but the active ingredient remains in the plant for a longer period at lower concentrations (Krupke et al., 2017; Magalhaes et al., 2009). During this period of insecticide degradation, many aphids survive these sublethal concentrations (Krupke et al., 2017; Magalhaes et al., 2009; Mccornack and Ragsdale, 2006) and might excrete honeydew contaminated with neonicotinoids that harm biological control agents.

Soybean aphid honeydew represents the main carbohydrate source for many beneficial insects in soybean fields (Dieckhoff et al., 2014; Heimpel et al., 2004; Lee et al., 2006) and increases their fitness when they feed on it (Dieckhoff et al., 2014; Lee et al., 2006; Tena et al., 2018; Wyckhuys et al., 2008). Two groups of biological control agents that commonly feed on honeydew are predators and parasitic wasps of aphids. The aphid-feeding predatory midge *Aphidoletes aphidimyza* is the most abundant dipteran predator in soybean fields and uses honeydew as food source and kairomone (Boulanger et al., 2019; Kaiser et al., 2007). The parasitic wasp *Aphelinus glycines* was purposefully introduced in United States to control the soybean aphid (Hopper et al., 2017), and *Aphelinus certus* was accidentally introduced and is now abundant throughout soybean growing areas of North America (Frewin et al., 2010; Kaser and Heimpel, 2018; Miksanek, 2020; Miksanek and Heimpel, 2019). Importantly, Frewin et al. (2014) showed that *A. certus* is susceptible to the neonicotinoids thiamethoxam and imidacloprid presented as soybean seed treatments in laboratory studies, and suggested that such seed treatments may limit the effectiveness of this parasitoid as a biological control agent of soybean aphid.

Here, we aimed i) to determine whether the neonicotinoid thiamethoxam from coated seeds reaches honeydew excreted by the soybean aphid *Aphis glycines* in a soybean crop; ii) to evaluate the toxicity for three species of biological control agents of honeydew obtained from plants whose seeds had been coated with neonicotinoids; and iii) to determine whether the use of fungicides in coated seeds synergizes the toxic effects of neonicotinoids via honeydew.

## 2. Materials and methods

### 2.1. Insect colonies

The soybean aphid was reared under laboratory conditions at the University of Minnesota on soybean plants grown from uncoated seeds following the methodology of Desneux et al. (2009) and this colony was

used to rear the parasitic wasps *A. certus* and *A. glycines*. Parasitic wasp colonies were maintained by placing approximately twenty individual parasitic wasps onto two soybean plants infested with aphids inside plexiglass rearing cages (30 × 35 × 40 cm) with ventilation provided by three 10 cm diameter holes that were covered with fine mesh. Parasitic wasp colonies were initiated weekly to provide sufficient females for the entire experiment. Parasitized aphids containing pupae ('mummies') of either parasitic wasp species were placed individually into glass vials (3 cm long × 0.8 cm diameter) plugged with cotton. Mummies were checked daily for emergence between 9:00 and 11:00 a.m. and were sexed after emergence. Newly emerged females were used for the experiments.

The predatory midge was obtained from Koppert Biological Systems. Pupae were introduced into rearing cages of the type described above and kept in climatic chambers until emergence. All insects were kept separately by species in growth chambers at 25 °C, 65% R.H. and 16:8 h L:D.

### 2.2. Study site and experimental design

The experiment was conducted at the University of Minnesota Agricultural Experiment Station, Saint Paul, U.S.A, between June and August 2019. The field experiment consisted of 24 soybean plots in a grid of 6 plots by 4 plots that were sown on June 19, 2019. Each plot was 2.5 × 2.5 m, and consisted of four soybean rows planted at a density of 35.6 seeds per m<sup>2</sup> (355,831 seeds per ha). Rows within each plot were separated by 0.76 m and plots were separated by 7.25 m. Buckwheat was planted and mown weekly between plots. Soybean and corn seeds coated with neonicotinoids were sowed in the same field the previous year, 2018.

We used a randomized complete block design of three different treatments each with eight replicates. Soybean seeds of the variety S14-B2X (Syngenta Crop Protection, USA) were uncoated or coated with either the insecticide thiamethoxam (Cruiser 5FS®, Syngenta) at a concentration of 0.0756 mg active ingredient per seed, or with the insecticide thiamethoxam in addition to the fungicides sedaxane, mepanipyrim (also called R-metalaxyl), and fludioxonil (CruiserMaxx Vibrance®, Syngenta) at a combined concentration of 0.0945 mg per seed for all active ingredients per seed, of which 0.0756 mg was thiamethoxam.

### 2.3. Plant infestation

Soybean plants were infested with the soybean aphid on July 16, 2019 (27 days after sowing, DAS). For this, we placed infested leaves from a laboratory colony with approximately 50 *A. glycines* of different instars on approximately ten plants per plot. Two plants per plot that were infested in this way were subsequently covered with exclusion cages to protect the infested leaves from natural enemies (Kaser and Heimpel, 2018). Exclusion cages consisted of a wire frame cage of 85 cm tall, and 35 cm × 35 cm square and were covered with a fine mesh (240 μm × 240 μm gaps).

### 2.4. Honeydew collection

Honeydew was collected in two temporal replicates. The first temporal replicate comprised honeydew collected on the following days: July 19, 2019 (+30 DAS), and every day from July 23 (+34 DAS) until July 26 (+37 DAS) inclusive. The second temporal replicate consisted of honeydew collected every day from July 30 (+41 DAS) to August 1 (+43 DAS) inclusive. To collect honeydew, Parafilm® squares of 10 cm × 10 cm were placed singly inside 14-cm Petri dishes inside the exclusion cage, and the cover of the Petri dish was modified with a fine mesh for ventilation (Fig. S1). A soybean leaf infested with 50–100 soybean aphids was inserted into the dish with the petiole passing through a hole in the side of the Petri dish in such a way that the infested leaf was

suspended above the Parafilm® (Fig. S1). The Parafilm® squares were left inside the Petri dishes in this manner for 24 h and stored at  $-20^{\circ}\text{C}$  until the honeydew was used in the bioassays described below.

### 2.5. Concentration of thiamethoxam in honeydew

We first estimated the amount of honeydew (i.e. the number of droplets) excreted by soybean aphids feeding on soybean plants following the methodology of Calvo-Agudo et al. (2019). The amount of honeydew produced by the soybean aphid per treatment, time replicate and plot was assessed by counting the total number of small (less than  $150\ \mu\text{m}\ \varnothing$ ), medium (between 150 and  $300\ \mu\text{m}\ \varnothing$ ), and large (more than  $300\ \mu\text{m}\ \varnothing$ ) honeydew droplets on each Parafilm® piece under a stereo microscope. The volume of each categorized droplet was estimated as  $(\frac{4}{3} \times \pi \times r^3) \times \frac{1}{2}$  where  $r$  is the radius of the droplet (Table S1). To ensure sufficient honeydew volume, we combined samples collected +30 DAS and +37 DAS for the first time replicate. For the second, we combined samples collected +41 DAS and +42 DAS. In total, we used honeydew samples from three treatments, seven to eight plots per treatment and from two time replicates to assess the presence of insecticide in the honeydew samples.

All droplets of honeydew from the same time replicate and plot were dissolved in 'Sample Diluent Buffer' (Imidacloprid ELISA, Microtiter Plate-kit, Abaraxis. Inc., Spain). Two hundred microliters of 'Sample Diluent Buffer' solution were pipetted onto the Parafilm® piece containing the honeydew droplets. The diluent solution and the honeydew droplets were stirred gently to dissolve the honeydew and then transferred into microcentrifuge tubes.

The presence and concentration of thiamethoxam in honeydew samples was estimated using an enzyme-linked immunosorbent assay for imidacloprid (ELISA-Imidacloprid, Microtiter Plate; Abraxis). This assay, although designed to detect imidacloprid, also detects clothianidin with 121% cross-reactivity, according to the manufacturer's specifications. Given that thiamethoxam is quickly metabolized to clothianidin in plants and insects and that the latter is the responsible for the insecticidal activity (Nauen et al., 2003; Tomizawa and Casida, 2005), we measured the presence and quantity of clothianidin in our samples as a proxy of that of thiamethoxam. All quantities were corrected considering the 121% cross-reactivity of clothianidin. This method allowed the quantification of very low amounts of insecticide, including potential residual contaminations from previous treatments in the experimental field (Masiá et al., 2013). In our assays, we detected the chemical in the samples coming from control treatments (average  $0.13\ \text{ng mL}^{-1}$ ; see Results). Hence, for the sake of accuracy, we corrected the values of all treatments by subtracting the average detection from the controls (Calvo-Agudo et al., 2019; Masiá et al., 2013). Negative values after the correction were converted to zero (Calvo-Agudo et al., 2019; Masiá et al., 2013).

### 2.6. Effect of honeydew on beneficial insects

To determine the effects of seed treatments on the beneficial insects' longevity, we fed adults of the predatory midge and parasitoids of genus *Aphelinus* with honeydew excreted by the soybean aphid that had fed on plants whose seeds had been untreated or coated either with thiamethoxam or with thiamethoxam and fungicides. For the predatory midge, we confined between 40 and 42 newly emerged and unfed female adults individually in 6.1 cm high and 2.8 cm diameter vials covered with damp cotton for each of the three treatments for a total of 122 individuals. For the parasitic wasps, we placed between 38 and 41 newly emerged unfed females individually into 3 cm high and 0.8 cm diameter glass vials covered with damp cotton for each of the treatments for a total of 116 *A. certus* and 123 *A. glycinis* individuals. Honeydew from each plot was used to feed between two and eight individuals of each species.

Parafilm® pieces with honeydew of each treatment were thawed, observed under a stereo microscope to check for the presence of honeydew and cut into pieces of different sizes to provide honeydew *ad libitum* (this was at least 10–15 and 25–30 droplets of different sizes for both parasitic wasp species and predator, respectively). Honeydew was renewed daily to avoid crystallization (Hogervorst et al., 2007). To confirm that all insects had received honeydew *ad libitum*, the presence of honeydew on the Parafilm® piece was checked again when it was replaced. If there was no honeydew remaining on the Parafilm®, the replicate was censored. Vials were kept in climatic chambers until the insects died and mortality was tabulated daily. The individuals from each treatment were fed on honeydew from six to eight plots of their corresponding treatments depending on the amount of honeydew available.

Climatic conditions for the predatory midge were  $25^{\circ}\text{C}$ , 80% relative humidity (RH) and 16:8 h light:dark (L:D) and for *A. certus* and *A. glycinis* were  $22^{\circ}\text{C}$ , 80% relative humidity (RH) and 16:8 h light:dark (L:D).

### 2.7. Statistical analysis

To analyze the difference in the concentration of thiamethoxam in the honeydew samples, we used a generalized linear mixed model with gamma distribution. The field plot was included as a random factor and treatment and time replicate as fixed factors. Non-significant factors were excluded from the final model. A Tukey post hoc test using the "lsmeans" package in R enabled pairwise comparisons between the concentrations found in the honeydew treatments. The toxicity of each honeydew treatment on the beneficial insect's survivorship was represented by Kaplan-Meier survivorship curves and analyzed by a Cox's Proportional Hazards for the predatory midge using the survival functions of the "Survival" package in R and by a log-rank test for both parasitic wasps. We censored those beneficial insects that escaped, died for other reasons, or had finished all the honeydew administered in one day (honeydew not *ad libitum*). For the predatory midge, we censored eighteen females out of 122 that escaped or died for other reasons and eleven out of 122 because they ran out of food during the trial. For *A. certus*, we censored nineteen females out of 116 that escaped or died for other reasons and five because honeydew had not been administered *ad libitum*. For *A. glycinis*, we censored 20 females out of 123 that escaped or died for other reasons and two because honeydew had run out during the trial. All tests performed were analyzed using the computer program R (version 3.3.2 for Macintosh).

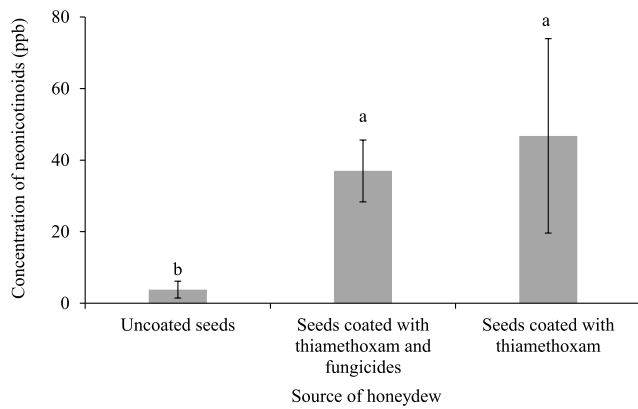
## 3. Results

### 3.1. Detection and quantification of neonicotinoids in aphid honeydew

The concentration of clothianidin, the derivate metabolite of thiamethoxam responsible for the insecticidal activity (Nauen et al., 2003; Tomizawa and Casida, 2005), was 9–11 times higher in honeydew excreted by aphids feeding on soybean plants whose seeds were coated either with thiamethoxam ( $46.76 \pm 27.17\ \text{ppb}$ ) or thiamethoxam and fungicides ( $36.98 \pm 8.66\ \text{ppb}$ ) than in honeydew from untreated plants ( $3.8 \pm 2.37\ \text{ppb}$ ) (GLMM,  $\chi^2_2 = 13.57$ ,  $P = 0.001$ ; Fig. 1 and Table S1). These concentrations of clothianidin were similar when they were collected 30–37 or 40–43 days after sowing the soybean (GLMM; days after treatment:  $\chi^2_1 = 1.18$ ,  $P = 0.27$ ) with no significant interaction between treatment and days after treatment ( $\chi^2_2 = 4.92$ ,  $P = 0.08$ ).

### 3.2. Longevity of beneficial insects

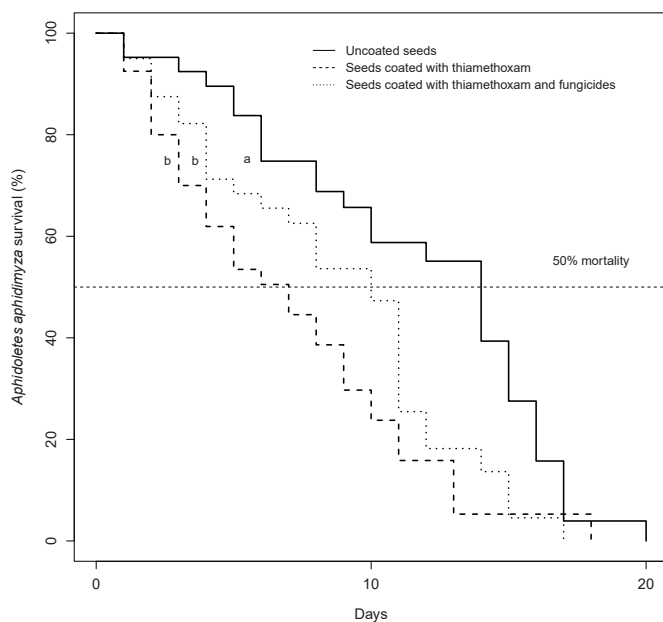
The longevity of female predatory midges that fed on honeydew produced by aphids feeding either on plants from seeds coated with thiamethoxam only or thiamethoxam plus fungicides was significantly shorter (median values 7 and 10 days, respectively) than of those fed on honeydew produced by aphids on untreated plants (median: 14 days)



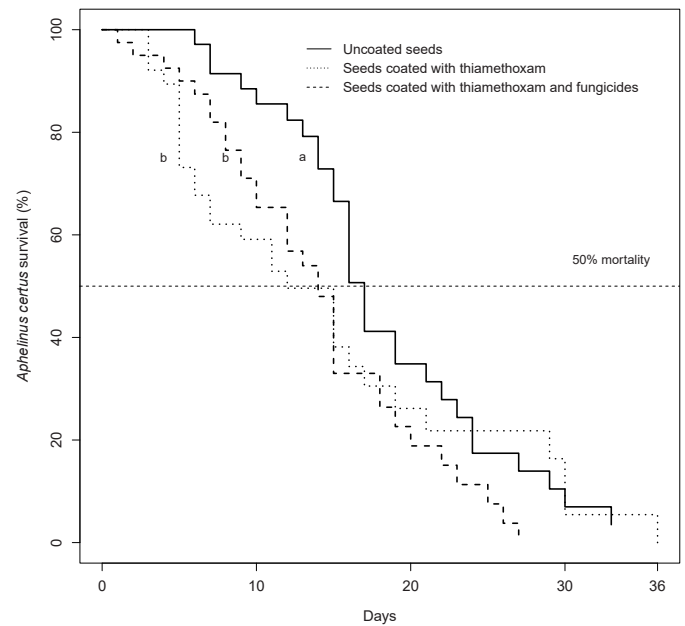
**Fig. 1. Neonicotinoid concentration in honeydew samples.** Concentration (mean ± SE) of clothianidin in honeydew excreted by *Aphis glycines* feeding on plants whose seeds were uncoated or had been coated either with thiamethoxam only or thiamethoxam and fungicides. Columns with different letters are significantly different from each other (GLM, based on gamma distribution,  $P < 0.01$ ; number of plots per treatment = 14 to 16).

(Cox's Proportional Hazards:  $X^2_2 = 12.69$ ,  $P = 0.002$ ) (Fig. 2).

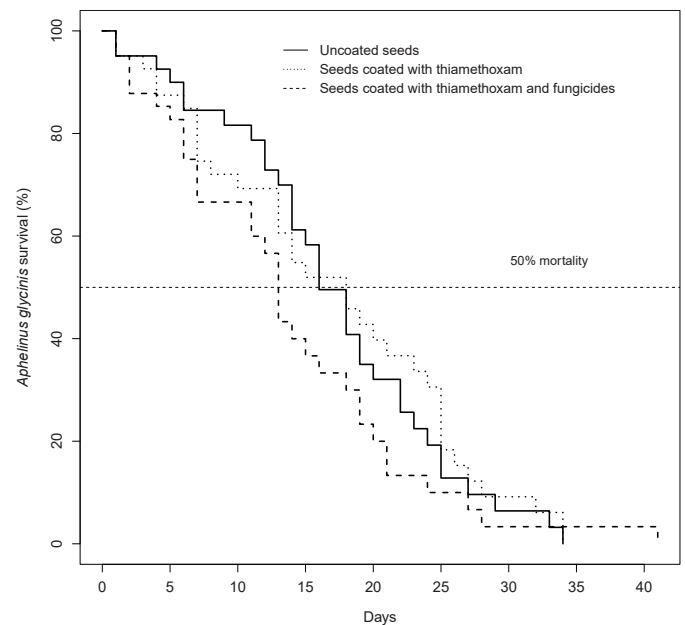
The longevity of female *A. certus* parasitoids feeding on honeydew produced by aphids feeding on plants whose seeds had been coated with thiamethoxam only or with thiamethoxam plus fungicides was also significantly shorter (median: 12 and 14 days respectively) than that of females fed on honeydew produced by aphids feeding on untreated plants (median: 17 days) (Log-rank Test:  $X^2_2 = 7.3$ ,  $P = 0.03$ ) (Fig. 3). The longevity of *A. glycines* females was not statistically different between the three honeydew types (median longevitys were 18, 16 and 13 days, respectively for females fed on honeydew excreted by aphids feeding on untreated plants, plants whose seeds were treated with thiamethoxam only or with thiamethoxam and fungicides; Log-rank Test:  $X^2_2 = 3.9$ ,  $P = 0.1$ ) (Fig. 4).



**Fig. 2. Survival curves of the predatory midge *Aphidoletes aphidimyza*.** Female midges were fed on honeydew excreted by soybean aphids feeding on plants whose seeds were uncoated or coated either with thiamethoxam only or thiamethoxam and fungicides.



**Fig. 3. Survival curves of the parasitic wasp *Aphelinus certus*.** Female wasps were fed on honeydew excreted by the soybean aphids feeding on plants whose seeds were uncoated or coated either with thiamethoxam only or thiamethoxam and fungicides.



**Fig. 4. Survival curves of the parasitic wasp *Aphelinus glycines*.** Female wasps were fed on honeydew excreted by the soybean aphids feeding on plants whose seeds were uncoated or coated either with thiamethoxam only or thiamethoxam and fungicides.

#### 4. Discussion

Our results demonstrate, for the first time, that neonicotinoids reach honeydew at concentrations that harm biological control agents when plants are grown from soybean seeds coated with neonicotinoids. The soybean aphid excreted honeydew contaminated with ~35–45 ppb of clothianidin when feeding on soybean plants that had been sown 30–43 days previously. We measured the concentration of clothianidin instead of thiamethoxam because the latter is quickly metabolized to

clothianidin in plants and insects (Nauen et al., 2003; Tomizawa and Casida, 2005), but some concentration of thiamethoxam might have remained in the plant after 30 days. Therefore, the total concentration of neonicotinoids might be higher than the reported here. In other crops, neonicotinoids derived from coated seeds have been detected in other plant-derived carbohydrate sources such as nectar, extrafloral nectar, or guttation fluids at concentrations as high as 1–8.6 ppb, 1–122 ppb, and 10 ppm, respectively (Girolami et al., 2009; Goulson, 2013; Jones et al., 2020; Rundlöf et al., 2015). However, these carbohydrate sources are absent in soybean agricultural landscapes where honeydew is the main, or only, carbohydrate source for biological control agents (Dieckhoff et al., 2014; Lee et al., 2006) and other beneficial insects.

We also detected low levels of clothianidin in honeydew excreted by aphids feeding on soybean plants from untreated seeds. This result might be explained by two non-exclusive reasons. First, plants might have absorbed residues from previous planting years as it was suggested by Krupke et al. (2017). In our study, soybean and corn seeds coated with neonicotinoids were sowed in some portions of the same field the previous year, 2018. Second, rainwater might have transported neonicotinoids from adjacent plots. Neonicotinoids are water soluble and plants take up only 2–20% of the neonicotinoid treatment with the remainder leaching into waterways (Sanchez-Bayo, 2014). In our study, it rained in 15 of the 31 days between sowing and honeydew collection (from June 19 until July 19, 2019) (US Climate Data, 2020). These rains caused intermittent runoff and, while we separated plots by 7 m, neonicotinoids might have moved from treated to untreated plots. However, this second reason seems less plausible because the field was flat and the soil sandy.

Honeydew contaminated with clothianidin from treated seeds reduced the longevity of two of the main biological control agents of the soybean aphid, the predatory midge and the parasitic wasp *A. certus*, when compared to the honeydew associated with non-treated seeds. Therefore, in this proof-of-concept study, we have demonstrated, for the first time, that neonicotinoids from coated seeds can reach honeydew and harm biological control agents. Further research will be necessary to evaluate the effects of honeydew contaminated with neonicotinoids derived from coated seeds on the disruption of biological control under field conditions. This research is likely unfeasible because many parameters of the biological control agents might be affected by neonicotinoids, e.g. immature parasitoids developing in contaminated hosts, contaminated prey for predators, biological control agents searching on plant surfaces contaminated with neonicotinoid dust particles, contaminated nectar from adjacent plants (Goulson, 2013; Krupke et al., 2012). On the other hand, honeydew excreted by aphids feeding on soybean plants from coated seeds did not result toxic to the parasitic wasp *A. glycinis* but reduced the longevity of *A. certus*. Previous studies have demonstrated that the toxicity of thiamethoxam is species-specific in parasitic wasps, even within the same genus (Cheng et al., 2018).

The three fungicides (fludioxonil, mefenoxam and sedaxane) used in the seed treatment did not synergize the toxicity of thiamethoxam. Fludioxonil is a phenylpyrrole fungicide used against a broad-spectrum of early-season pathogens that has limited systemic properties (Camargo, 2016). Therefore, it was not expected to contaminate honeydew. Instead, sedaxane and mefenoxam are systemic fungicides from the pyrazoles and phenylamides groups, respectively. Mefenoxam is one of the most commonly used products in soybean targeting *Pythium* spp., *Phytophthora* spp. and other plant pathogens of the order Peronosporales (Monkiedje et al., 2007), while sedaxane has a broader spectrum of activity (Zeun et al., 2013). To the best of our knowledge, only one study has evaluated the toxicity of mefenoxam and thiamethoxam on beneficial insects (Camargo, 2016). This study found no adverse effects on worker honeybees mortality and biological control agents when mefenoxam was administered alone via oral and contact exposure. In contrast, when mefenoxam was combined with thiamethoxam, unclear effects on honeybee mortality and no adverse effects for biological control agents were observed (Camargo, 2016).

## 5. Conclusions

This study demonstrates that the soybean aphid survives the seed-coated treatment and excretes honeydew that contains neonicotinoids derived from coated seeds. As explained in the introduction, soybean is not the only crop, whose seeds are commonly coated with neonicotinoids, and support a diverse group of aphids and other hemipterans that are resistant to neonicotinoids or tolerant to concentrations that degrade over time (De Ribeiro et al., 2018; van Leeuwen et al., 2010). These hemipterans likely also excrete honeydew contaminated with neonicotinoids rendering the honeydew toxic for biological control agents. Therefore, the prophylactic use of seeds coated with neonicotinoids may disrupt biological control through contaminated honeydew, increasing both the costs for growers and the environmental impacts of these major crops. Further studies are necessary to corroborate our results in other major crops.

Nowadays, only the European Union (which represents less than 4% of the global agricultural land) has banned the application of neonicotinoids after a risk assessment report carried out by the European Food Safety Authority. This risk assessment, as well as others carried out by other environmental agencies, did not consider honeydew as route of exposure to beneficial insects. This route is especially relevant because environmental agencies are now evaluating the use of neonicotinoid coated-seeds in crops that are harvested before the flowering period, without considering that these plants can hold phloem-feeding herbivores that excrete contaminated honeydew as we demonstrate here under field conditions. Therefore, honeydew should be included in future environmental risk assessments as a route of exposure to neonicotinoids applied as seed coating.

## Author contributions

M.C.-A., J. D, G. E. H., M.D. and A.T. contributed to the design of research; M.C.-A. and J.D. performed the research; J.G.-C., contributed new reagents/analytic tools; M.C.-A., and A.T. analyzed data; All co-authors wrote the paper.

## 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.

## Acknowledgments

We thank Syngenta Crop Protection for providing the seeds for the experiments. We acknowledge the research assistants Sarah Wood, Sierra Baum, and Amber Johnson who helped enormously with field and laboratory work. This research was partially funded by an INIA project (Project No. RTA2017-00095), an “Europa Excelencia” project (EUR2020-112293), the Conselleria d’Agricultura, Pesca i Alimentació de la Generalitat Valenciana, a grant from the University of Minnesota Rapid Agricultural Response Program, and the Minnesota Agricultural Experiment Station, United States. J.G.C was supported by the Spanish Ministry of Economy and Competitiveness, Ramón y Cajal Program (RYC-2013-13834), Spain. M.C-A was recipient of grant from INIA (CPD2016-0085), Spain.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.envpol.2021.117813>.

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