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The effect of top predator cues and an insecticide on prey choice of a generalist mesopredator

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ABSTRACT

Natural enemies of pests are affected by the use of chemical substances in agriculture, which can have lethal and various sub-lethal effects and disrupt their biocontrol service. In this work, I investigated how the pesticide Quasin may affect the adaptive prey selection of the spider *P. cespitum* (Philodromidae) of variously sized caterpillars *Operophtera brumata* as a response to the cues of the ant *Lasius fuliginosus*. The results show that the probability of prey capture by *P. cespitum* decreased with prey size. There was a trend that suggests that the foraging behaviour of *P. cespitum* was affected by the ant cues. These results also suggest that the natural herbicide Quassin did not affect the foraging behavior of the philodromids. Therefore, it seems to be a promising biopesticide that can be used in tandem with the natural enemies to supress insect pests.

Keywords: Biological control; Natural enemy; Pesticide; Generalist predator.

INDEX:

1. INTRODUCTION

Natural enemies, such as spiders, provide important biocontrol services by reducing arthropod pests (e.g. Riechert & Bishop, 1990; Suenaga & Hamamura, 2015; Cotes et al., 2018). But pesticides can severely affect their biocontrol services via lethal and various sub-lethal effects (Flint & Dreistadt, 1998; Deng et al., 2006). Pesticides can reduce the ability of spiders to adaptively respond to their environment such as to the presence of their own enemies (Wrinn et al., 2012). But the effect of pesticides on the adaptive response of pests' natural enemies is understudied.

The predation pressure exerted by spiders on pests depends, to the large extent, on their prey choice (Michalko et al., 2019). The prey choice influences predator density as well as their functional responses to pest (Toft, 1999; Smout et al., 2010). The prey choice is thought to be adaptive and it should change depending on the presence of an enemy (Houtman & Dill 1998). The foragers under the threat should select such prey that reduces the risk of predation although it is less rewardable (Houtman & Dill 1998). The ability to adaptively alter the prey choice may maintain viable spider populations in agroecosystems but pesticides can disrupt this ability.

Here is investigated how the pesticide Quasin may affect the adaptive prey selection of *P. cespitum* of variously sized caterpillars as a response to the cues of the ant *Lasius fuliginosus*. It is expected that the cues of the top predator will alter the prey acceptation of *P. cespitum*. In the presence of the ant cues, *P. cespitum* will select the prey if it is more easily subdued even though less energetically profitable. On the contrary, it is expected that *P. cespitum* exposed to pesticides does not change its prey selection since it does not detect the presence of predator cues and / or the differences in prey size.

2. OVERVIEW

2.1. Biological control

In recent decades, elevated awareness of the impacts of pesticide use on the environment and human health have resulted in efforts to reduce reliance on chemical controls (Desneux et al., 2019). Many countries have instituted more stringent regulation of pesticide manufacture, registration and use, thereby increasing the cost, and decreasing the availability of these tools (Wyckhuys et al., 2019). In many cases, the pests themselves have indicated the need for change, with pesticide resistance now a common reality in many weeds, insects and diseases (Douglas & Orr, 2019).

For this reason, they have begun to use agrarian techniques such as biological control, which is a component of an integrated pest management strategy (Douglas & Orr, 2019). It is defined as the reduction of pest populations by natural enemies and typically involves an active human role (Wyckhuys et al., 2019).

There are three broad and somewhat overlapping types of biological control: classical biological control (introduction of natural enemies to a new locale), augmentation and conservation of natural enemies (Douglas & Orr, 2019). Each of these techniques can be used either alone or in combination in a biological control program (Desneux et al., 2019).

The conservation biological control is a sustainable form of pest management, which promotes the natural enemies of pests in the agroecosystems (Begg et al., 2017). It includes supplying food sources, diversification of plants and habitats, a reduction in the cultivation intensity, and enhancing complexity of the landscape (Begg et al., 2017; El-Wakeil, 2017).

Although conservation biological control is one of the most effective and viable options to control mites, insect pests and weed pests (Barbosa, 1998), pest suppression or improvement in crop yields is not always obtained (Begg et al., 2017)

2.2. Generalist predators and biological control

A generalist predator is an organism that attacks, kills, and feeds on a variety of similar organisms (Flint & Dreistadt, 1998).

Spiders are known as natural enemies and are very important in agroecosystems (Flint & Dreistadt, 1998), they are ubiquitous in every terrestrial ecosystem (Animales y animales, 2019). Some species are epigeal, i.e., they live on the ground, while others are arboreal, i.e., they live in trees (Cotes et al., 2018). All spiders are predators and prey on many small-medium sized insects (Flint & Dreistadt, 1998). Some families, such as the wolf spiders, do so through active hunting, while others, such as the web-weaving spiders, do so through passive hunting, with their webs. Because many species spend the winter, they can help reduce the number of preys at the beginning of the agricultural season (Canna, 2019).

The predation pressure exerted by generalist predators on pests depends largely on predator densities and functional responses (Solomon, 1949; Sinclair et al., 1998). Both characteristics are heavily influenced by their prey choice, as this determines their fitness and capture rate of pest (Toft, 1999, Smout et al., 2010, Schmidt et al., 2012).

The prey chosen by a spider depends on the interaction between the features of the spider, such as the size of the spider and the hunting strategy (Michalko et al. 2019). It also depends to a large extent on the characteristics of its prey and environmental conditions (Michalko et al. 2019). There are studies that indicates that the presence of predators might affect the spider prey choice (Riechert & Hedrick 1993; Michalko & Pekár 2014). However, it is unknown whether it is caused through flexible change in behaviour due trait-mediated effect or through selective predation on certain behavioural phenotype.

2.2.1. Trait mediated effect and adaptive response to enemies

Predators not only eat prey, but they can also cause changes in prey behavior, morphology, and physiology as prey tries to avoid predators (Werner & Peacor 2003, Schmitz et al., 2004; Miner et al., 2005). This mechanism is called indirect interaction mediated by traits (Abrams 1995, Peacor & Werner 1997) or non-consumptive indirect effect (Abrams 2007). For example, the presence of predators can reduce the foraging of prey (Huang & Sih 1991).

The predation pressure exerted by these predators on pests depends largely on predator densities and functional responses (Solomon, 1949; Sinclair et al., 1998). Both characteristics are heavily influenced by their prey choice, as this determines their suitability and rate of pest capture (Toft, 1999, Smout et al., 2010, Schmidt et al., 2012).

For prey selection, spiders use vibration, tactile and chemical signals. Inter-specific chemical signals, i.e., between species (kairomones) are used by prey to avoid predators, to locate prey in the case of predators (Koivula & Korpimaki, 2001).

The prey chosen by a spider depends on the interaction between the features of the spider, such as the size of the spider and the hunting strategy. It also depends to a large extent on the characteristics of its prey, and environmental conditions. But there are studies that sign that the cues of predators might affect the spider prey choice (Riechert &Hedrick 1993; Michalko & Pekár 2014), although these signs are still unknown.

Therefore, spiders are known as natural enemies and are very important in agroecosystems, but pesticides can seriously affect biological control, damaging natural enemies (Flint & Dreistadt, 1998).

2.3.Disruption of biological control by chemical pesticides

Intensive use of pesticides is common practice to reduce pest populations in agroecosystems (Bommarco et al., 2011). However, the broad-spectrum pesticides have negative effects also on the non-target organisms such as pollinators and pest natural enemies via lethal and various sub-lethal effects (Aveling, 1977; Vickerman, 1988; Desneux et al. 2007; Harsimran, & Harsh, 2014). The application of some insecticides can even lead to more severe pest attacks because of the natural pest control exerted by enemies is reduced (Pickett & Patterson 1953, Huffaker & Kenneth 1956, Ripper 1956, Dempster 1968, Bommarco et al. 2011, Harsimran, & Harsh 2014). This is because pesticides can eliminate natural enemies or change their behaviour and life-history parameters (Pullen et al., 1992, Frampton, 1999, Shaw et al., 2006, Pedersen et al., 2002; Babczynska et al., 2006; Deng et al., 2006). The recognition of the negative effects of pesticides on the non-target organisms led to a development of specific pesticides that should only be toxic to the target organisms, be biodegradable, and eco-friendly (Rosell et al., 2008; Harsimran, & Harsh, 2014). Nevertheless, even the so-called specific pesticides can still have various negative effects on non-target organisms and their effects need to be tested.

One mechanism, how the pesticides can affect the biocontrol services of the generalist predators, is alteration of their prey choice (Lurling & Scheffer 2007, Klaschka 2008, Petcharad et al. 2019). Pesticides can affect the prey choice of generalist predators in various ways. In some cases, pesticides can impair the sensory systems (Wrinn et al., 2012; Leccia et al., 2016). Due to the deterioration of the sensory system, predators may be unable to distinguish between various types of prey. On the other hand, pesticides may also increase the voracity of generalist predators, which may lead to a lower degree of prey selectivity (Riechert 1991, Guedes & Cutler, 2014, Niedobova et al., 2016, Michalko & Pekar, 2017; Petcharad et al., 2018).

2.4.Aims and hypotheses

Here I used the spider *Philodromus cespitum* as the mesopredator and the ant *Lasius fuliginosus* as the top predator to study how the pesticide Quassin may affect the adaptive prey choice of *P. cespitum* as a response to the cues of the ant. We investigated the prey choice between variously sized caterpillars depending on the presence of ant cues and exposure to the pesticide in two full factorial design.

I expected that the cues of the ant will alter the prey choice of *Philodromus*. In the presence of the ant cues, the philodromids will select the prey that is more easily subdued but less energetically profitable because it would devote more energy / attention to enemy vigilance than to prey capture. On the contrary I expected that the philodromids exposed to pesticides does not change its prey choice since it does not detect the presence of predator cues and / or the differences in prey size.

3. MATERIALS AND METHODS

3.1.Studied system

The spider used for the experiment was *Philodromus cespitum* (Walckenaer, 1802) (Philodromidae) (mean ± SD carapace size = 0,9087 ± 0,32242 mm), the ant *Lasius fuliginosus* (Latreille, 1798) (Formicidae) as the spider enemy, and the caterpillar of *Operophtera brumata* (L. 1758) (Geometridae) (mean ± SD body size = 0,39 ± 2,42445 mm) as the spider prey. These species naturally co-occur in fruit orchards and surrounding semi-natural habitats (Korenko & Pekár 2010; Michalko & Pekár 2015).

P. cespitum is dominant spider species in fruit orchards in Central Europe and it is known to prey on caterpillars but to avoid ants (Bogya et al. 1999; Michalko & Pekár 2015). *L. fuliginosus* occurs in various habitats and the workers climb trees and shrubs to care for the aphids. The jaws are relatively weak, but small insects can be taken as food. Their enemies are repelled by anal aromatic secretions (Waldén, 1964). This ant species induce emigration in various spider species (Mestre et al. 2014). Larvae of *O. brumata* are general feeders and will defoliate a variety of tree and shrub species (Elkinton et al., 2015). They are detrimental defoliator of several host trees including Garry Oak (*Quercus garryana*), aspen and poplar (*Populus* spp.), birch (*Betula* spp.), and Bigleaf Maple (*Acer macrophyllum*) as well as some agricultural crops, such as blueberries and cranberries, and fruit orchards (Broadley, 2018).

3.2.Collection and maintenance of arthropods

I collected he arthropods in an area located in north of Brno, near the Faculty of Regional Development and International Studies, Mendel University of Brno at the end of April. (Figure 1 and 2) They were collected by beating shrubs and trees above the beating tray (Figure 3).

Spiders were put in Eppendorf tubes (1.5 ml) individually while ants, which came from one nest, and caterpillars were placed in plastic jars together. A total of 100 individuals of *P. cespitum*, 50 individuals of *L. fuliginosus*, and 100 caterpillars of *O. brumata* were collected.

To standardize the hunger level, the spiders were let to starve for five days. Then I fed each spider individual with one aphid three day before the experiments.

Figure 1: Collection zone (Google Maps, 2019)

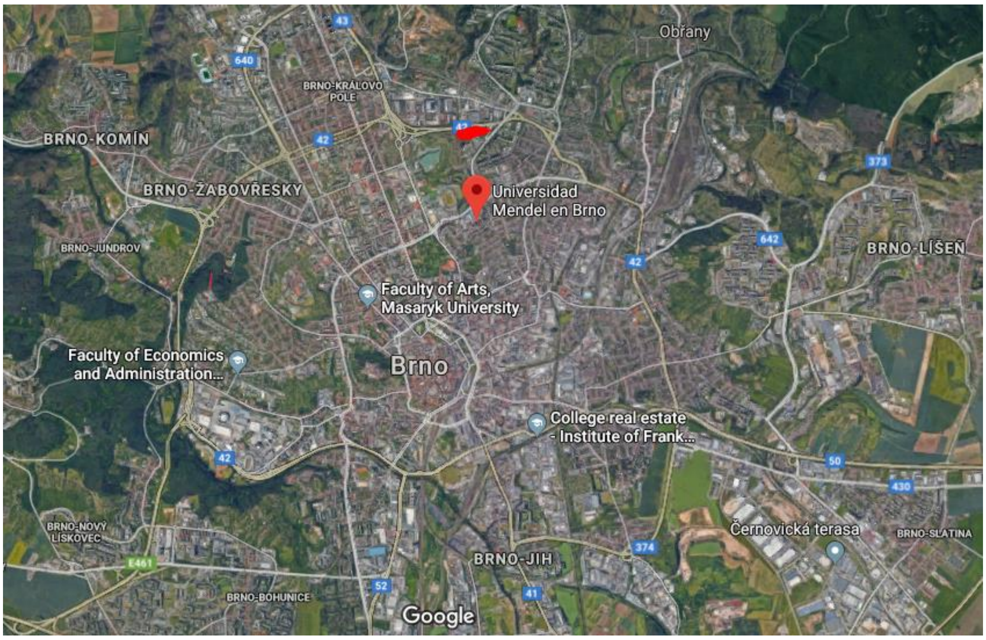

Figure 2: Collection zone in Brno (Google Maps, 2019)

Figure 3: Beating tray used to collect the spiders (Mocholí C., 2019)

3.1.Pesticide

For the pesticide I used the plant extract from *Quassia amara*. This biopesticide is used against various insect pests such as aphids and sawflies in fruit orchards. It is a food and contact poison with cytotoxic and repelling effects (Morant 2013). The active ingredient of the pesticide is Quassin (Figure 4). It is a white, bitter and crystalline substance from the quassinoid family that when used as pesticide create a protective barrier on the plant that repels pests by contact, ingestion and systemic action (Morant M., 2013).

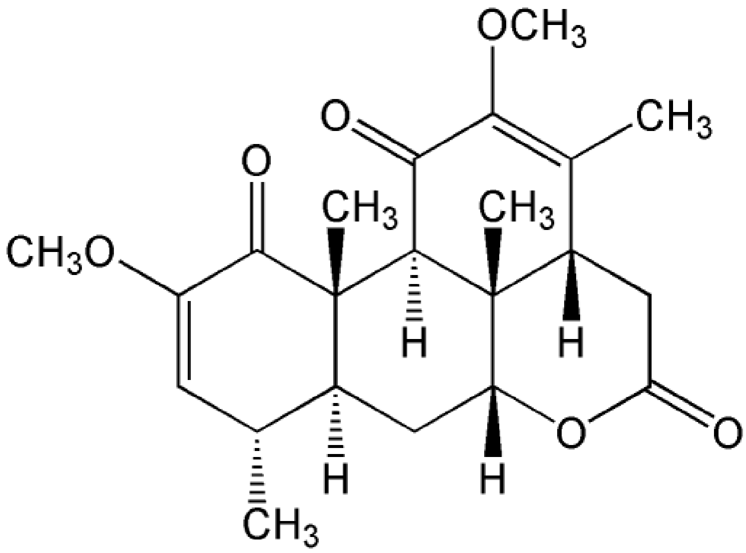

Figure 4: Structure of the diterpene lactone Quassin. Created in ChemDraw by User: Walkerma, March 2006. Structure taken from Shing, T. K. M.; Jiang, Q; Mak, T. C. W. J. Org. Chem. 1998, 63, 2056-2057.

One of the advantages of this pesticide is that it is a natural substance, so its use is thought to be not as harmful to the environment as conventional chemical pesticides (Porcurna, 2011). For this reason, there is growing interest in its potential uses for the sustainable management of farms (Morant, 2013).

Quassin is extracted from the infusion of wood chips. Care should be taken when using it, as it is irritating to the eyes and mucous membranes (Dominguez, 2019).

For the experiments, I used the recommended concentration (0.06%) for field application. The concentration that the pesticide was made was 50 grams per litter of water, because 25 grams of it were putted in half a litter of distilled water. Then I boiled the mixture for 2 hours, and after 2 hours the half of the mixture, it means 250 ml, and It was diluted with 1250 ml of distilled water.

3.3.Experimental design

For the experiment I rolled 50 filter papers into tubes and submerge them in the insecticide for 2 seconds. They were left to drip off and then dry for 1 hour at room temperature.

I used, as a control, 50 papers with distilled water doing the same process that with the papers with pesticide

After that, I introduced the spiders into the tubes and closed them to maximize their contact with the residues. The spiders were exposed to the pesticide residues or water control for 45 minutes.

The 50 ants were put in 50 plastic jars for the 50 spiders that would be exposed to the ant cues and after one hour they were removed from the plastic jars.

The experimental design was full 2 x 2 factorial design, i.e.: control - predator cue / control - no cue / insecticide - predator cue / insecticide - no cue. Each treatment had 25 replicates (N = 100).

After exposure to the insecticide or water control, philodromids were placed individually in plastic jars with or without top predator cues.

Before introduction of a caterpillar to a spider, I measured the caterpillar body length and spider carapace width size. After that, I introduced one *Operophtera brumata* to each philodromid. The philodromid and prey were left to interact for one hour. After that, it was checked to see if the philodromid killed the prey.

3.4.Statistical analyses

To evaluate the predation trials, generalized linear models (GLM) with binomial error structure and logit link (GLM-b) were used because the data had Bernoulli's distribution (Zuur et al. 2015). The adequate model was selected with the information–theoretic approach (Zuur et al. 2015). Twelve candidate competitive models including the null model (Table 1) were built. The optimal model was selected based on Akaike weights (ω_i) (Zuur et al. 2015). The data were not over-dispersed.

4. RESULTS

The most optimal model contained only the effect of body size ratio between caterpillar and spider (Table 1; Figure 5). However, the second and third most optimal model contained also the effect of enemy (Table 1). The enemy cues seemed to reduce the probability of prey acceptance and so reduce the overall prey size that the philodromids would accept (Figure 5B). The models containing the effect of Quassin had relatively poor explanatory power (Table 1) and the effect of the pesticide seem to be therefore minimal.

Table 1: Comparison of the binomial GLMs predicting the acceptance probability of caterpillar of *Operophtera brumata* by the spider *Philodromus cespitum*. ΔAIC and ωi refer to AIC difference and AIC weights, respectively, Enemy – presence of cues of the ant *Lasius fuliginosus*, Pesticide – philodromids exposed to residues of Quassin, and Size ratio – caterpillar length to carapace width of philodromids. The three most optimal model are highlighted in grey.

Model	Linear predictor	d.f.	AIC	Δ AIC	ω_i
1	Null	1	127.954	26.400	0.000
2	Enemy	2	127.253	25.699	0.000
3	Pesticide	2	129.131	27.577	0.000
4	Size ratio	2	101.554	0.000	0.320
5	Enemy + Size ratio	3	102.499	0.945	0.200
6	Enemy * Size ratio	4	103.349	1.794	0.130
7	Pesticide + Size ratio	3	103.386	1.831	0.128
8	Pesticide * Size ratio	4	105.198	3.643	0.052
9	Enemy + Pesticide + Size ratio	4	104.339	2.784	0.080
10	Enemy + Pesticide * Size ratio	5	106.073	4.519	0.033
11	Pesticide + Enemy * Size ratio	5	105.116	3.561	0.054
12	Pesticide * Enemy * Size ratio	8	110.714	9.160	0.003

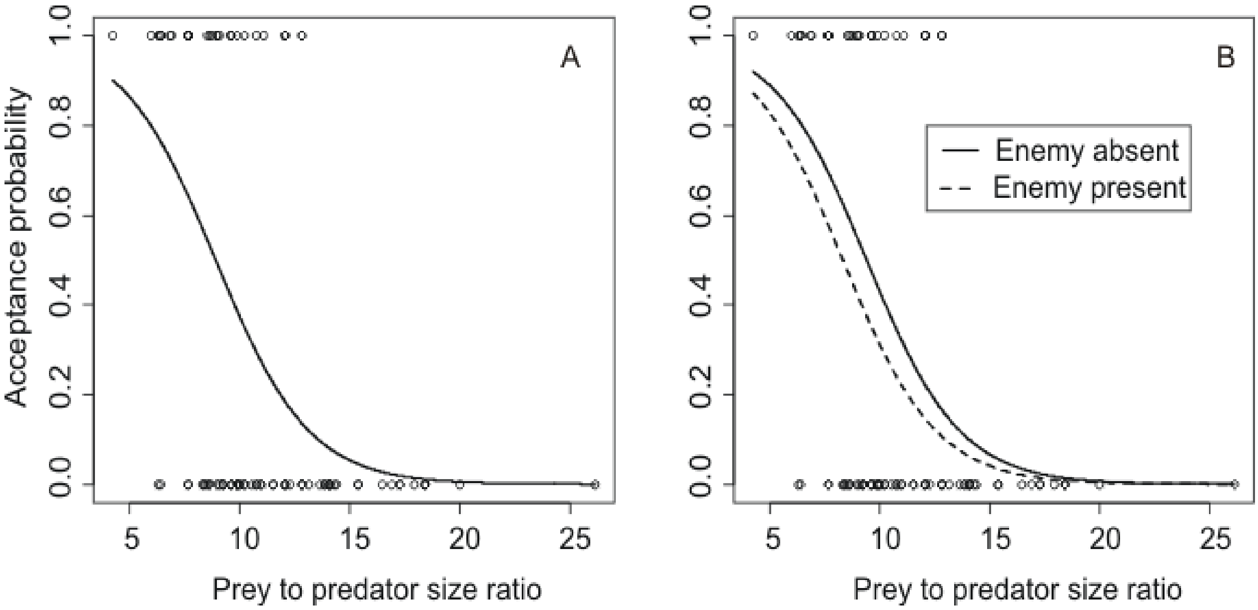

Figure 5: Acceptance probability of caterpillar of *Operophtera brumata* by the spider *Philodromus cespitum*.

The size ratio was computed between body length of caterpillar and carapace width of spider. Estimated relationship of the best (A; $y = \frac{1}{1+ e^{-4.213+0.472x}}$) and of the second best model (B; $y_{enemy\ absent} = \frac{1}{1 + e^{-4.446+0.472x}}$, $y_{enemy\ present} = \frac{1}{1 + e^{-3.396+0.472x}}$) based on Akaike weights are shown (Table 1).

5. DISCUSSION

Here, I investigated how the pesticide Quassin influences the predatory behavior of the spider *Philodromus cespitum*. I found that the insecticide does not affect the prey acceptance of the spider and the predatory activity.

The results of this experiment suggest that *P. cespitum* is not sensitive to Quassin and therefore the pesticide studied seems to be harmless to this spider.

5.1.Prey size relation

As we can see in the experiment, the acceptance of the prey is affected mainly by the size ratio between *P. cespitum* and the prey, *O. brumata*. Because the philodromids did not kill to the caterpillars with a relation of size too big for them. This may be due to the fact that they cannot simply overcome the prey it is very big or if the prey is very small for them they would need more energy to kill the prey than they would obtain from the nutritional value of eating it, this is because the additional energy expenses that are invested in capturing and subduing the prey that will not provide the same amount of the energy used to capture or more can, therefore, affect the capacity of the philodromids (Stephens et al., 2007).

5.2.Predator cues

On the other hand, as compared in other studies (Wrinn. et al., 2012), the effect of the enemy also seems to affect the choice of prey, but to a lesser extent than the relationships between body size between the mesopredator and the prey, because, as we can see, (Figure 5B) the spiders that were exposed to the presence of enemy signals reduced the size of the prey they accepted.

This may be due to the fact that part of the energy they would use to capture prey would be necessary to remain alert to the enemy's signals, so they prefer to choose prey that imply a lower investment to subdue it or faster consumption.

These results suggest that the response to the signals of *Lasius fuliginosus* with herbicide are detectable by the spider, so this pesticide does not affect and does not modify the behavior of *P. cespitum* in the presence of the ants.

5.3.Pesticide effect

There was no detectable response of *P. cespitum* to the herbicide, since other studies with *P. cespitum* showed that another kind of pesticide could alter the chemical signals and behavior of the spider (e.g. Petcharad et al., 2018, Cloyd, 2012, Stark, & Banks, 2003, Brown et al., 2014; Benamu et al., 2013)

On the other hand, the pesticide Quassin does not seem to have an effect on *P. cespitum*, because it seems that the Philodromids continued capturing the same number of *Q. brumata* in the presence of the pesticide than without the presence of the pesticide. This means that a short exposure of the spider to the pesticide does not alter the behavior of the spider or its selection of prey size.

This may be due to the fact that it is a natural insecticide and supposedly not harmful to the environment (Porcuna, 2011).

6. CONCLUSION

The results show that the probability of prey capture by philodromids decreased with prey size. There was a trend that suggests that the foraging behaviour of *Philodromus cespitum* was affected by the cues of *Lasius fuliginosus*. These results also suggest that the natural herbicide Quassin did not affect the foraging behavior of the philodromids. Therefore, it seems to be a promising biopesticide that can be used in tandem with natural enemies to supress insect pests. Nevertheless, additional tests with other organisms are necessary for the firm conclusions.

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