Determination of the functional response in the orb-weaving spider *Araneus diadematus* (Araneae: Araneidae) according to insecticide type

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Abstract

1. Orb-weaving spiders are abundant predators in agroecosystems and serve as key natural enemies for pest control. However, studies have demonstrated that many insecticides can negatively affect the predatory behaviours of spiders when exposed to sublethal concentrations, thus disrupting their biocontrol potential and subsequent ecosystem dynamics. Understanding how insecticides impact spiders is, therefore, of great importance.

2. This study investigated the effects of two conventional insecticides (thiamethoxam and deltamethrin), and a common biopesticide (neem oil) compared to a tap water control on the functional response of a common orb-weaver *Araneus diadematus*.

3. Spiders were collected from the wild and maintained under laboratory conditions in containers (20 x 20 x 5cm) to allow for web production. Spiders were then exposed to one of the four treatments and *Drosophila melanogaster* were added to the webs as prey at densities of 1, 3, 5, 10, 20, and 40, with the number of consumed prey quantified after 16-hours to determine the functional response.

4. Overall, *A. diadematus* exhibited a type II functional response when exposed to the control, thiamethoxam and neem oil treatments, with comparable consumption rates, search coefficients and handling times. This contrasted to deltamethrin treated spiders which exhibited type III functional responses and a lower consumption rate of prey compared to the control.

5. This study demonstrates that deltamethrin, unlike thiamethoxam and neem oil, is capable of negatively affecting the biocontrol potential of *A. diadematus*. However, further research is required to fully understand the impact insecticides have on the predatory behaviours of orb-weaving spiders.

Keywords: Spider, natural enemy, biocontrol, agroecosystem, insecticide, functional response

Introduction

Synthetic insecticides are used extensively in agriculture to reduce the damaging effects of insect pests (Ware & Whitacre, 2004; Benamú et al., 2007; Aktar et al., 2009). However, despite their benefits, studies have estimated that when applied, up to 95% of insecticides do not reach their target organism and effectively enter the environment as a toxic pollutant (Aktar et al., 2009; Fernandes et al., 2010; Oberemok et al., 2015). This consequently threatens non-target species across aquatic and terrestrial systems, including agriculturally beneficial invertebrates (van der Werf, 1996; Aktar et al., 2009; Sharma et al., 2019). Predatory arthropods, or natural enemies, that prey upon pest species are of particular importance as they are an invaluable resource for agroecosystems as biological controls, especially for Integrated Pest Management (IPM) programs (Fernandes et al., 2010; Zeng et al., 2018). The depletion of such natural enemies, through the effects of insecticide exposure, can therefore lead to a resurgence of pests, or secondary pest outbreaks (Fernandes et al., 2010). This can result in significant environmental and economic damage (Fernandes et al., 2010), as well as a decreased biodiversity that further hinders the stability and overall function of the agroecosystem (Balvanera et al., 2006; Cardinale et al., 2012).

Insecticides can affect natural enemies, as well as other non-target and target organisms, through lethal or sublethal exposure (Walker, 2004; Fernandes et al., 2010; Pekár, 2012). Although sublethal exposure does not result in death, it can alter the physiology, development and behaviours of arthropods (Samu & Vollrath, 1992; Desneux et al., 2007; Pekár, 2012). Whilst this may appear to be less detrimental to natural enemy populations, the effects are often comparable and perhaps of more importance than lethal exposures (Hanna & Hanna, 2013), especially when considering the greater rate of encounter in nature (Tahir et al., 2019; Ricupero et al., 2020). This has consequently led to an increasing research focus on their impacts on agriculturally beneficial fauna (Desneux et al., 2007; Sánchez-Bayo, 2011; Mužinić & Želježić, 2018). In addition, alternatives to harmful synthetic formulations, such as selective, biologically and botanically derived insecticides, or biopesticides, are progressively replacing the non-selective synthetics (Kogan, 1998; Benamú et al., 2007; Singh et al., 2019). Despite the ecological and environmental benefits of biopesticides and selective insecticides, the potential negative effects on natural enemies are still largely unknown, particularly in a sublethal context (Chowański et al., 2014; Haddi et al., 2020).

Spiders have been documented as the most abundant and important group of predatory invertebrates throughout many terrestrial ecosystems, including agroecosystems (Nyffeler & Benz, 1987; Samu & Vollrath, 1992; Nyffeler & Birkhofer, 2017). Spider populations naturally exist as an assemblage of several species, and as generalist predators they collectively stabilise invertebrate communities, regulating the density of other predators and herbivores (Branco & Cardoso, 2020; Milano et al., 2021), and even suppress pest populations, acting as an essential biological control (Sunderland, 1999; Sharma et al., 2013). Although the sublethal effects of insecticides on spiders have been reported to be species-specific (Pekár, 2002; 2012), any disturbance in one guild or species group infers implications for the dynamics and function of the entire assemblage. Moreover, studies have previously demonstrated the adverse impact insecticides can have on spider population dynamics and diversity, with very few at the species level (Pekár, 2012; Pedro et al., 2020). Therefore, for the preservation of the spider assemblage and the associated ecosystem services they provide, it is necessary to understand the species level impacts of insecticides (Riechert & Lawrence, 1997; Sharma et al., 2013).

Orb-weaving spiders are a particularly useful guild for sublethal studies of insecticides as the webs are a fixed record of complex behaviours that are governed by internal and external factors (Samu & Vollrath, 1992; Hesselberg, 2015). Many studies have utilised the orb-web and its ease of analysis to aid in understanding the effects of insecticides and other neurotoxins on associated web-building behaviours (Hesselberg & Vollrath, 2004; Blackledge, 2011; Albín et al., 2014). Orb-weavers are also known to play a crucial role as natural enemies in agroecosystems, and in some cases, they constitute the largest guild within the assemblage of spiders (Uetz et al., 1999; Sharma, 2014; Poolprasert & Jongjitvimol, 2014). Orb-weavers, and other web-building spiders, provide added benefits as they can reduce pest populations through both direct consumption and wasteful killing (Mukhtar et al., 2018). This is mostly exhibited by web-building spiders and occurs when more prey is killed than is consumed (Maloney et al., 2003), further adding to the importance of orb-weaving spiders in agroecosystems (Sunderland, 1999; Maloney et al., 2003).

Stability in a predator-prey system, at species and assemblage level, is crucial for the suppression and maintenance of pest populations and is achieved through density-dependent responses by the predator (Sharma et al., 2013). A useful tool for measuring such density-dependence and a predator's impact is the quantification of the functional response, which is the relationship between prey density and consumption rate (Holling, 1959). Functional responses are widely used to evaluate the effectiveness of natural enemies for the biological control of pests (Smith, 1984; Mukhtar et al., 2018; Lacava et al., 2021), and distinguishing between the various types of response (i.e. type I, II or III) is important owing to the differences in their contribution to prey population stability (Hassell et al., 1977). There are three basic types of functional responses; type I is defined by a linear relation between prey density and the number of prey consumed, with a constant intake rate; type II occurs when the number of prey consumed declines with an increasing prey density until satiation and is influenced by the time taken to locate, capture and process food (i.e. search coefficient and handling time); type III is defined by a sigmoidal relation whereby the consumption rate accelerates at low prey densities, then decreases at higher densities eventually reaching an asymptote (Holling, 1959; Dawes & Souza, 2013). Spiders typically exhibit a type II functional response (Smith, 1984; Řezáč et al., 2010), however, there are some reported instances of spiders exhibiting a type I and a type III response (Jeschke et al., 2003; Butt & Xaaceph, 2015; Benhadi-Marín et al., 2019). Observing the functional response of spiders exposed to insecticides can help determine their potential implications on their function as natural enemies. Although it is known that insecticides can negatively affect the predatory efficacy of numerous spider species (Pekár, 2012; Michalko & Košulič, 2016; Butt et al., 2019), there have been few that have investigated how insecticides affect the predatory behaviours of orb-weavers specifically, despite their evident significance in agroecosystems (Smith, 1984; Uetz et al., 1999; Řezáč et al., 2010).

This study, therefore, investigated the effects of two non-selective synthetic insecticides and one biopesticide, compared to a control treatment, on the functional response of *Araneus diadematus* towards a generic prey resource of fruit flies *Drosophila melanogaster*. The focal species is a common orb-weaving spider found in various habitats throughout Britain (Foelix, 2011; Bee et al., 2019) as well as much of Europe and North America (Smith, 1984). *A. diadematus* has been reported as an abundant species in field margins and other agroecosystem habitats (Sloggett, 2010), feeding on a range of insect species, including important pests from the families Diptera, Heteroptera, Hymenoptera and Coleoptera (Ludy, 2007; Sloggett, 2010). Moreover, *A. diadematus* is a reliable species that has been used for behavioural studies as it can be easily maintained and readily produces an orb-web in a laboratory setting (Smith, 1984; Zschokke & Herberstein, 2005). The abundant nature and compatibility with laboratory-based research made *A. diadematus* an ideal organism for this study.

Methods

Spider collection and maintenance

Immature *A. diadematus* (body length: 3.8 ± 0.6mm [mean ± SD]) were hand collected from small shrubs and trees in a grassland habitat in North Ayrshire, Scotland (55°38'24.4"N; 4°42'22.6"W), from July to September 2021. Individual spiders were placed within plastic vials (3cm length; 1cm diameter) and returned to the laboratory where species confirmation and measurements were made using a stereomicroscope. Individual spiders were transferred to a container comprised of a wooden frame (20 x 20 x 5cm) with plastic panels (20 x 20 x 0.1cm) placed on either side. The spiders were then held for a 3-day acclimation, starvation and web construction period. Thereafter, spiders were kept for a further 3-days during which time any spiders that produced an orb web were used for a feeding trial (see below). Spiders were misted with water daily and were maintained at 24 ± 3°C room temperature and 60 ± 5% relative humidity, with a natural photoperiod of approximately 14:10hrs (L:D).

Insecticide preparation and exposure

Four treatment groups were used in this study consisting of a tap water control, the two synthetic insecticides, neonicotinoid thiamethoxam (Axoris® Bug Attack; 1% w/w), and pyrethroid deltamethrin (Provanto® Ultimate Bug Killer; 1.5% w/w), and the biopesticide neem oil (Garten Glück; 1.8% w/w). All insecticides were applied at the manufacturers' recommended field dosage for foliar application according to the instructions provided and the correct dilutions were achieved using tap water. When a spider had spun an orb web, it was carefully removed from its container without damaging the web and was randomly allocated, and subsequently exposed, to one of the four treatments following a similar method of tarsal exposure by Řezáč et al. (2010). A piece of filter paper (11cm diameter) was submerged in the respective solution for 5-seconds and then dried at room temperature for 30-minutes. The filter paper was then rolled and placed into a test tube (9cm length; 2cm diameter), followed by a spider, and the test tube was plugged with cotton wool to prevent escape. Spiders were exposed to the treatment for 30-minutes and afterward were removed from the test tube and placed onto their web once the allocated density of prey had been added (see below). The method of tarsal exposure could be considered a limitation of the present study as orb-weaving spiders are more likely to encounter pesticide residue via their webs (Rhoades & Stoddard, 2021), however, this method is well established and has been demonstrated to be an acceptable form of exposure (e.g. Michalko & Košulič, 2016; Petcharad et al., 2018; Řezáč et al., 2019a)

Functional response trials

The prey used in this experiment were laboratory cultured Turkish glider fruit flies, *Drosophila melanogaster* (Advanced Husbandry; Highcroft Industrial Estate, Portsmouth), which were kept in the same conditions as the spiders. During a feeding trial, individual spiders exposed to the different insecticide treatments were randomly presented prey at six densities (1, 3, 5, 10, 20, 40; N=4 per experimental treatment). Fruit flies to be used in feeding trials were removed from the culture containers and placed in a freezer for 3-minutes to temporarily immobilise the flies before they were added onto the web. After this, the spider was removed from the holding vial and placed onto the hub of the web. Experiments were initiated between 16:00 - 18:00 each day and spiders were permitted 16hrs to interact with the prey.

Once the experiment was complete, all remaining flies were removed from the container and web using forceps and observed under a stereomicroscope to determine if they had been killed or left untouched. Flies that were wrapped in silk, regardless of whether they remained situated on the web or not, and the remains of consumed flies were categorised as killed prey. The remaining flies that were not killed by the spider were categorised as untouched prey. Control treatments were single replicates of each prey density without spiders present to confirm the deaths of flies in the experiment could be attributed to predation.

Statistical analysis

All statistical analyses were conducted using R v4.1.1 (R Core Team, 2020). Differences in overall prey consumption according to insecticide treatment were analysed using a generalised linear model (GLM) with a quasi-Poisson error distribution and log link. For the analysis, prey consumption was pooled across the density categories. Effect sizes in the model were inferred using F-tests via analysis of deviance. Statistical significance was inferred at the 95% confidence level.

Functional responses were first assessed via logistic regression to determine the shape of the response (i.e. type I, II or III) by analysing the proportional prey consumption as a function of initial prey density. Here a significantly negative first-order term indicates a type II response whereas a significantly positive first-order term followed by a significantly negative second-order term indicates a type III response (Juliano, 2001). Once the general form of the response had been determined, models were fit using a flexible model that induces a scaling exponent q to allow for a continuum of shapes between type I, II and III to be described (Barrios-O'Neill et al., 2015):

$$Ne = N0\ (1\text{-}\exp\ (b\ N0^{q}\ (h\ Ne - T)))$$

Where Ne is the number of prey eaten, N0 is the initial prey density, b is the search coefficient, h is the handling time, q is the scaling exponent and T is the total time available. Where type II responses occur, $q = 0$, and functional responses become increasingly type III in form when $q > 0$. In order to compare functional responses of spiders from different insecticide treatments, 95% confidence intervals were fitted around functional response curves by non-parametrically bootstrapping the datasets ($n = 2000$). As this allows the functional response to be considered at the population-level as opposed to the sample-level, differences in functional responses were made on the basis of confidence interval divergence across prey densities (Pritchard et al., 2017).

Results

There was high survival of prey in control treatments (>90%), thus experimental deaths were attributed to spider predation. Moreover, there were no spider mortalities observed in the insecticide treatments during the course of the experiment. There were no statistical differences in the overall consumption among the treatments studied (GLM: $F_{3,93} = 2.61$, $p = 0.08$), however, there was a clear reduction in the numbers of prey consumed in the deltamethrin treatment compared to the other treatments (Figure 1). Logistic regression indicated type II functional responses for *A. diadematus* exposed to the control, neem oil, and thiamethoxam treatments and a type III response when exposed to deltamethrin (Table 1, Figure 1). When considering the 95% confidence limit overlaps, the search coefficient was found to be similar among the treatments that produced a type II response, however, it was found to be lower (albeit obtained without certainty) in the deltamethrin treatment (Table 2, Figure 2a). Spiders exposed to deltamethrin had a greater handling time than all of the other treatments except neem oil, whereas the treatments that produced type II responses had similar handling times (Table 2, Figure 2b).

Discussion

No direct mortality was observed in this study, but we found a significant decrease in the consumption rate and a marked, though non-significant, decrease in the average number of prey consumed by spiders exposed to deltamethrin compared to the other treatments. Moreover, *A. diadematus* was found to exhibit a type III functional response when exposed to this insecticide compared to the type II functional response that was observed when exposed to the control, neem oil and thiamethoxam treatments. These differences were also reflected in changes to handling times. Although the behavioural effects of these insecticides reported here may be subtle at the species level, the potential implications for the dynamics of the population and assemblage of spiders, and thus the agroecosystem functioning, are significant.

This study found that the pyrethroid deltamethrin had the most notable effect on *A. diadematus*. The negative effects of pyrethroids in spiders have been well documented (Pekár, 2012) where they are known to cause significant mortalities as well as impair locomotion at sublethal concentrations (Ware & Whitacre, 2004; Pekár, 2012). Like all synthetic pyrethroids, deltamethrin affects the nervous systems of invertebrates by holding open the voltage-gated sodium channels found in neuron membranes (Ware & Whitacre, 2004; Garcia, 2011). As a result of the constant excitation of the neuron, deltamethrin causes paralysis and death at lethal concentrations (Ware & Whitacre, 2004; Garcia, 2011). Paralysis can also occur at sublethal doses, for shorter durations, as well as impaired locomotion, uncoordinated mobility, and convulsions (Garcia, 2011). For example, a decrease in walking speed when exposed topically to deltamethrin has been observed in the erigonid spider *Oedothorax apicatus* (Everts et al., 1991) and exposure to λ-cyhalothrin left only the first two pair of forelegs functional in *Philodromus* spiders (Michalko & Košulič, 2016). In spiders, locomotion is key for all aspects of foraging and associated predatory behaviours (Chmiel et al., 2000), including the initial construction of the orb-web and the detection, attack, and capture of prey (Chmiel et al., 2000; Pekár, 2012). Such impacts have been demonstrated in the orb-weaving spider *Larinioides sclopetarius* whereby the web-building behaviour and web geometry were negatively affected after exposure to deltamethrin (Lengwiler & Benz, 1994). Similar effects have been reported by other researchers that likewise inferred pyrethroid exposure impairs coordination of the legs, implicating the locomotion necessary for web construction (Samu & Vollrath, 1992) and predation (Benamú et al., 2013). Although the web structure, and associated web-building behaviours, were not directly observed in this study, it is plausible that the orb-web suffered adverse alterations or was perhaps left unamended when damaged after the spiders were exposed to the pyrethroid deltamethrin (i.e. Samu & Vollrath, 1992; Benamú et al., 2010; 2013). Such effects on the web structure could have further compromised the predatory efficacy of *A. diadematus* (Benamú et al., 2013; Tew et al., 2015), however, this cannot be said with certainty. In this study, *A. diadematus* also displayed increased prey handling times and consumed less prey overall compared to the other treatments, which is likely a result of the impaired mobility and capture speed due to deltamethrin exposure. It is also possible that paralytic effects of the pyrethroid impaired function of the chelicera and other mouthparts, preventing *A. diadematus* from efficient consumption of prey and increasing the handling times, an observation that has been made in other studies (Řezáč et al., 2010; Michalko & Košulič, 2016).

A noteworthy result from the deltamethrin exposed spiders was the shift to a type III functional response. This is a finding that has been observed previously by Heidari et al. (2015) in the parasitoid *Encarsia formosa* when exposed to the pyrethroid fenpropathrin, implicated by nervous system disruption. Although information concerning the neurological effects of pyrethroids on spiders is limited (Benamú et al., 2010), it is probable that predator-prey interactions in this study were also disrupted as a result of an impaired nervous system (Benamú et al., 2010; Tahir et al., 2019). For example, the prolonged nerve stimulation caused by deltamethrin exposure has been reported to distort kairomone detection and increase the arrestment response upon detection, as documented by Delpuech et al. (2005) when studying the effects of deltamethrin on the parasitoid wasp *Leptoplilina heterotoma*. The increased time for a response or reaction upon detecting prey could, therefore, implicate the search coefficient of *A. diadematus* resulting in the observed lower consumption rates (Figure 2c). Other pyrethroids have also been known to interfere with sensory receptors and detection of prey in spiders via kairomones which can reduce the consumption rates in some species (Desneux et al., 2007). Orb-weavers are sit-and-wait predators that rely on vibratory signals generated by prey caught in their webs to initiate their capture and immobilisation (Klärner & Barth, 1982; Masters et al., 1986; Hesselberg, 2015). Therefore, deltamethrin could have potentially interfered with other sensory receptors in *A. diadematus*, such as those necessary for vibratory detection and olfaction. If the vibratory and olfactory senses necessary for the detection of prey were distorted in *A. diadematus* then they may not recognise the prey (Petcharad et al., 2018; Tahir et al., 2019). This could be especially important when detection of prey at low densities is reduced resulting in a lower consumption rate, and potentially a type III functional response. In addition, exposure to deltamethrin may have decreased the probability of interaction between predators and prey because of induced paralysis and impaired mobility in the spiders. Therefore, even if prey detection by *A. diadematus* was unaffected, the escape rate of prey from the web is still likely to have been greater than fully functional spiders. A similar consequence has been observed in windy conditions that impact locomotion and slow the capture speeds of orb-weaving spiders and also lead to an increased rate of prey escape from the webs (Turner et al., 2011). In our study, there was a shift to a type III response as a result of deltamethrin exposure, but further research investigating all aspects of predatory behaviour by *A. diadematus* exposed to this insecticide is required.

The other synthetic insecticide in this study, thiamethoxam, surprisingly did not invoke a change in the functional response of *A. diadematus* compared to the control, and a type II response was observed. Neonicotinoids, likewise, act on the nervous system, although they block the postsynaptic nicotinic acetylcholine receptors (Maienfisch, et al., 2001; Ware & Whitacre, 2004; Tomizawa & Casida, 2005). These receptors bind with the enzyme acetylcholinesterase, which is essential for removing the neurotransmitter acetylcholine from the synapse (Ware & Whitacre, 2004; Ghosal, 2018). This results in overstimulation of the neuron, causing uncontrolled and uncoordinated movement, tremors and eventually death at high concentrations (Ware & Whitacre, 2004). Now, although mortalities have been previously reported in Lycosidae and Oxyopidae spider populations in response to thiamethoxam (Nazli & Butt, 2020; Lacava et al., 2021), spiders are generally considered less susceptible to neonicotinoids (Song et al., 2009), which aligns with our findings. Notwithstanding, studies have documented reduced consumption rates in thiamethoxam exposed spiders along with impacts to locomotory behaviours, which again has implications for predation (Butt et al., 2019; Korenko et al., 2019; Řezáč et al., 2021). There is also evidence of species-specific effects in terms of how exposure occurs and Řezáč et al. (2019a) notably reported that the mode of uptake produced different results when studying the effects of various neonicotinoids in *Oedothorax apicatus* and *Phylloneta impressa* spiders. Here, dorsal applications resulted in more mortalities than tarsal contact, and when *P. impressa* was exposed to lower concentrations through tarsal contact there were no recorded mortalities (Řezáč et al., 2019a). In a similar study, the neonicotinoid imidacloprid significantly reduced the predation rate of *Philodromus cespitum* only through dorsal contact, whereas tarsal exposure had no significant effect (Řezáč et al., 2019b). Korenko et al. (2019) likewise noted a decrease in prey consumption when *Pardosa agrestis* were provided with neonicotinoid-treated flies. The form of exposure is evidently an important determinant for the effects of neonicotinoids in spiders and it is apparent that tarsal exposure is the least detrimental, as opposed to dorsal application or direct ingestion. In this study, tarsal contact was also the selected method of exposure, which could potentially explain the negligible effects of thiamethoxam on *A. diadematus*. In this respect, it is important to consider that orb-weaving spiders are more likely to encounter insecticide residue through their webs and direct ingestion (Pekár, 1999; Rhoades & Stoddard, 2021), however, in the field orb-weaving spiders could still uptake insecticide residue through tarsal contact, either from the droplets collected on the orb-web, or residues on the surrounding substrate (i.e. leaves or branches). Moreover, studies have demonstrated that some pesticides repel spiders (Pekár & Haddad, 2005; Pekár & Beneš, 2008) and cause them to abandon their webs (Samu & Vollrath, 1992). This could ultimately increase the likelihood of migrations across contaminated substrata and thus, the incidence of pesticide exposure via tarsal contact. Future studies should, however, aim to implement such methods of exposure that closely relate to field conditions.

The neem oil treatment also had no effect on the functional response of *A. diadematus* compared to the control with spiders exhibiting a type II response with similar search coefficients and handling times. Neem-based products are readily available to control a range of pest species at varying life stages and are therefore a valuable insecticide for agriculture (Campos et al., 2016; Adhikari et al., 2020). Neem oil, in particular, is the most widely used neem-based product and it has many pesticidal functions due to a complex composition of over 200 biologically active compounds facilitating the anti-oviposition, anti-feed and repellent properties (Stark, 2013; Campos et al., 2016; Gupta et al., 2017; Adhikari et al., 2020). Many studies have determined that neem oil, along with other selective and botanical insecticides, should have a negligible effect on spiders and other non-target arthropods in an agroecosystem (Mansour & Nentwig, 1988; Stark, 2013). There is evidence however of an effect on behaviours in certain species, such as reduced consumption rates in the crab spider *Philodromus cespitum* (Řezáč et al., 2010). The results from the present study correspond with research indicating that neem-based products have very minimal sublethal effect on spiders (Mansour & Nentwig, 1988; Stark, 2013), and are likely safer for the environment than non-selective, synthetic insecticides such as deltamethrin (Chowański et al., 2014; Haddi et al., 2020). More studies evaluating potential behavioural impacts of predation in spiders are imperative for the complementary use of neem in agroecosystems.

In conclusion, deltamethrin was the only insecticide to significantly affect the functional response and associated parameters of *A. diadematus*, resulting in a reduced predatory efficiency and subsequent biocontrol potential. Overall, the results were consistent with other studies, with a type II functional response that has been widely reported in numerous spider species (Smith, 1984; Řezáč et al., 2010; Félix-Oliveira et al., 2022), alongside the observed detrimental impact of deltamethrin and the marginal effects of neem oil. Although neonicotinoids are typically considered harmful to non-target organisms in an agroecosystem and have been shown to negatively impact spiders, thiamethoxam produced results akin to the control treatment in this study implying that they are of little concern to *A. diadematus*. Further research regarding the effects of different forms of exposure is essential, particularly relating to field-accurate routes of exposure. However, it is worth emphasising that we only investigated one aspect of prey capture in this study. Many pesticides also affect the structure of the orb web, which again will have an effect on the efficiency of prey capture (Samu & Vollrath, 1992; Pasquet et al., 2016). Our results on the impact of neem oil align with many studies demonstrating that it is an effective insecticide that can be used in combination with the spider assemblage for complementary biological control (Mansour & Nentwig, 1988; Stark, 2013). However, further research is required to fully understand the potential behavioural impacts of insecticides, particularly biopesticides, to ensure effective implementation of insecticides and spiders for biological control in agroecosystems, and to further preserve the surrounding environment and associated ecosystem dynamics.

Acknowledgements

The authors would like to acknowledge the Association for the Study of Animal Behaviour who supported the work reported here through a project scholarship grant awarded to Kieran Boyd.

Author contributions

KMB and MEA conceived the study. KMB performed the experiments, collected and analysed the data with contributions from MEA and TH. KMB wrote the initial manuscript which was edited by MEA and TH.

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Table 1. Parameter estimates from logistic regression analyses of proportion of prey consumed against initial density for *A. diadematus* exposed to the control, neem oil, deltamethrin, and thiamethoxam treatments. Values for 1st and 2nd order terms are presented with p-values.

Treatment	**Intercept (p-value)**	**1st order (p-value)**	**2nd order (p-value)**	**FR type**
Control	1.199 (<0.001)	-0.019 (<0.05)	-	II
Neem Oil	1.566 (<0.001)	-0.048 (<0.001)	-	II
Deltamethrin	-2.069 (<0.001)	0.167 (<0.01)	-0.004 (<0.001)	III
Thiamethoxam	1.331 (<0.001)	-0.027 (<0.01)	-	II

Table 2. Search coefficients (b) and handling times (h) along with the scaling coefficient (q) from the functional response model parameters of *A. diadematus* when exposed to the control, neem oil, deltamethrin, and thiamethoxam treatments. Values for parameters are presented with p-values.

Treatment	**b±SE (p-value)**	**h±SE (p-value)**	**q±SE (p-value)**
Control	1.458 ± 0.217 (<0.001)	0.014 ± 0.006 (<0.05)	Fixed at 0
Neem Oil	2.495 ± 0.008 (<0.001)	0.050 ± 0.008 (<0.001)	Fixed at 0
Deltamethrin	0.049 ± 0.044 NS	0.088 ± 0.015 (<0.001)	1.145 ± 0.426 (<0.01)
Thiamethoxam	1.603 ± 0.255 (<0.001)	0.021 ± 0.006 (<0.001)	Fixed at 0

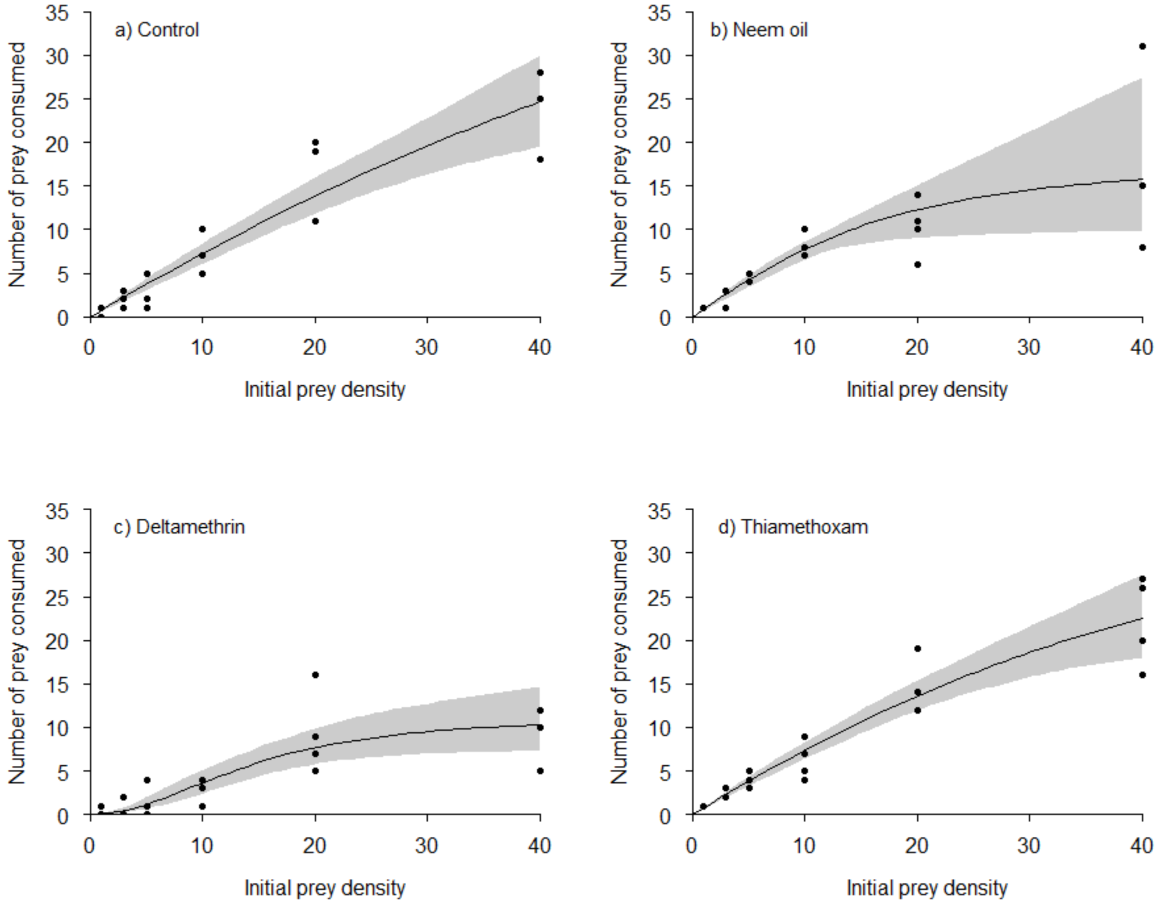

Figure 1. Functional responses of *A. diadematus* towards *D. melanogaster* when exposed to a) control, b) neem oil, c) deltamethrin, and d) thiamethoxam. Solid lines represent the fitted model curve and shaded areas represent 95% confidence intervals calculated by non-parametric bootstrapping. Raw data points are provided; however, some values overlap.

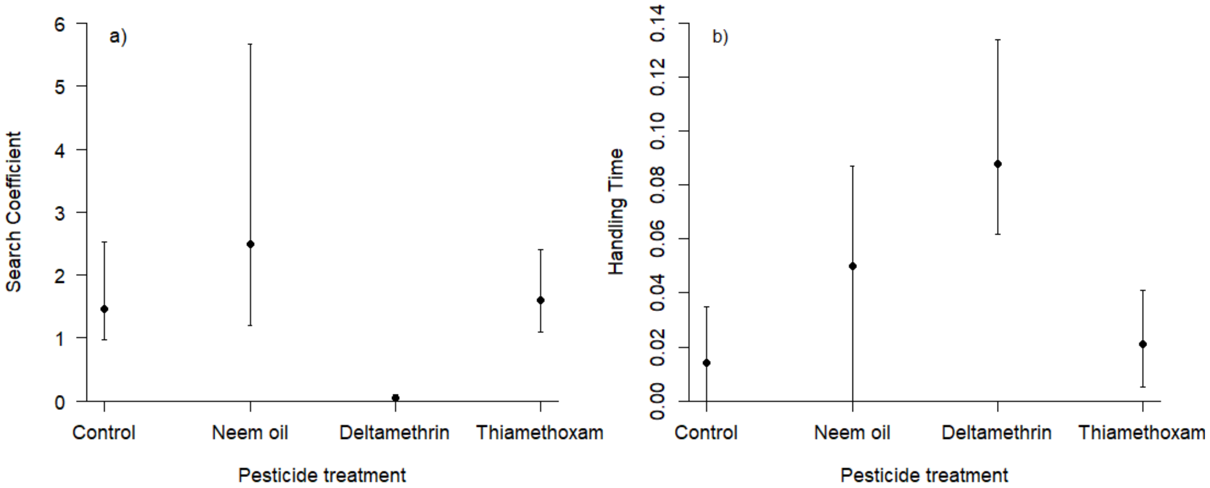

Figure 2. Functional response model parameters for the a) search coefficient and b) handling time of *A. diadematus* when exposed to the control, neem oil, deltamethrin and thiamethoxam treatments over a 16-hour period. Solid dots represent the mean values, and the error bars are presented with 95% confidence intervals calculated by non-parametric bootstrapping (n=2000).