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

3  
4 Kieran M. Boyd<sup>1\*</sup>, Thomas Hesselberg<sup>2</sup>, Mhairi E. Alexander<sup>1</sup>

5  
6 <sup>1</sup> Institute for Biomedical and Environmental Health Research, School of Health and Life Sciences,  
7 University of the West of Scotland, Paisley, Scotland, PA1 2BE, United Kingdom

8 <sup>2</sup> Department of Zoology, University of Oxford, South Parks Road, Oxford, OX1 3PS, United Kingdom

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10 \* Corresponding author: kieran\_boyd123@yahoo.com

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50 **Abstract**

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

56  
57 2. This study investigated the effects of two conventional insecticides (thiamethoxam and deltamethrin), and  
58 a common biopesticide (neem oil) compared to a tap water control on the functional response of a common  
59 orb-weaver *Araneus diadematus*.

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61 3. Spiders were collected from the wild and maintained under laboratory conditions in containers (20 x 20 x  
62 5cm) to allow for web production. Spiders were then exposed to one of the four treatments and *Drosophila*  
63 *melanogaster* were added to the webs as prey at densities of 1, 3, 5, 10, 20, and 40, with the number of  
64 consumed prey quantified after 16-hours to determine the functional response.

65  
66 4. Overall, *A. diadematus* exhibited a type II functional response when exposed to the control, thiamethoxam  
67 and neem oil treatments, with comparable consumption rates, search coefficients and handling times. This  
68 contrasted to deltamethrin treated spiders which exhibited type III functional responses and a lower  
69 consumption rate of prey compared to the control.

70  
71 5. This study demonstrates that deltamethrin, unlike thiamethoxam and neem oil, is capable of negatively  
72 affecting the biocontrol potential of *A. diadematus*. However, further research is required to fully understand  
73 the impact insecticides have on the predatory behaviours of orb-weaving spiders.

74  
75 **Keywords:** Spider, natural enemy, biocontrol, agroecosystem, insecticide, functional response

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97 **Introduction**

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

111  
112 Insecticides can affect natural enemies, as well as other non-target and target organisms, through lethal or  
113 sublethal exposure (Walker, 2004; Fernandes et al., 2010; Pekár, 2012). Although sublethal exposure does not  
114 result in death, it can alter the physiology, development and behaviours of arthropods (Samu & Vollrath, 1992;  
115 Desneux et al., 2007; Pekár, 2012). Whilst this may appear to be less detrimental to natural enemy populations,  
116 the effects are often comparable and perhaps of more importance than lethal exposures (Hanna & Hanna,  
117 2013), especially when considering the greater rate of encounter in nature (Tahir et al., 2019; Ricupero et al.,  
118 2020). This has consequently led to an increasing research focus on their impacts on agriculturally beneficial  
119 fauna (Desneux et al., 2007; Sánchez-Bayo, 2011; Mužinić & Želježić, 2018). In addition, alternatives to  
120 harmful synthetic formulations, such as selective, biologically and botanically derived insecticides, or  
121 biopesticides, are progressively replacing the non-selective synthetics (Kogan, 1998; Benamú et al., 2007;  
122 Singh et al., 2019). Despite the ecological and environmental benefits of biopesticides and selective  
123 insecticides, the potential negative effects on natural enemies are still largely unknown, particularly in a  
124 sublethal context (Chowański et al., 2014; Haddi et al., 2020).

125  
126 Spiders have been documented as the most abundant and important group of predatory invertebrates  
127 throughout many terrestrial ecosystems, including agroecosystems (Nyffeler & Benz, 1987; Samu & Vollrath,  
128 1992; Nyffeler & Birkhofer, 2017). Spider populations naturally exist as an assemblage of several species, and  
129 as generalist predators they collectively stabilise invertebrate communities, regulating the density of other  
130 predators and herbivores (Branco & Cardoso, 2020; Milano et al., 2021), and even suppress pest populations,  
131 acting as an essential biological control (Sunderland, 1999; Sharma et al., 2013). Although the sublethal effects  
132 of insecticides on spiders have been reported to be species-specific (Pekár, 2002; 2012), any disturbance in  
133 one guild or species group infers implications for the dynamics and function of the entire assemblage.  
134 Moreover, studies have previously demonstrated the adverse impact insecticides can have on spider population  
135 dynamics and diversity, with very few at the species level (Pekár, 2012; Pedro et al., 2020). Therefore, for the  
136 preservation of the spider assemblage and the associated ecosystem services they provide, it is necessary to  
137 understand the species level impacts of insecticides (Riechert & Lawrence, 1997; Sharma et al., 2013).

138  
139 Orb-weaving spiders are a particularly useful guild for sublethal studies of insecticides as the webs are a fixed  
140 record of complex behaviours that are governed by internal and external factors (Samu & Vollrath, 1992;  
141 Hesselberg, 2015). Many studies have utilised the orb-web and its ease of analysis to aid in understanding the  
142 effects of insecticides and other neurotoxins on associated web-building behaviours (Hesselberg & Vollrath,  
143 2004; Blackledge, 2011; Albín et al., 2014). Orb-weavers are also known to play a crucial role as natural  
144 enemies in agroecosystems, and in some cases, they constitute the largest guild within the assemblage of  
145 spiders (Uetz et al., 1999; Sharma, 2014; Poolprasert & Jongjitvimol, 2014). Orb-weavers, and other web-

146 building spiders, provide added benefits as they can reduce pest populations through both direct consumption  
147 and wasteful killing (Mukhtar et al., 2018). This is mostly exhibited by web-building spiders and occurs when  
148 more prey is killed than is consumed (Maloney et al., 2003), further adding to the importance of orb-weaving  
149 spiders in agroecosystems (Sunderland, 1999; Maloney et al., 2003).

150  
151 Stability in a predator-prey system, at species and assemblage level, is crucial for the suppression and  
152 maintenance of pest populations and is achieved through density-dependent responses by the predator (Sharma  
153 et al., 2013). A useful tool for measuring such density-dependence and a predator's impact is the quantification  
154 of the functional response, which is the relationship between prey density and consumption rate (Holling,  
155 1959). Functional responses are widely used to evaluate the effectiveness of natural enemies for the biological  
156 control of pests (Smith, 1984; Mukhtar et al., 2018; Lacava et al., 2021), and distinguishing between the  
157 various types of response (i.e. type I, II or III) is important owing to the differences in their contribution to  
158 prey population stability (Hassell et al., 1977). There are three basic types of functional responses; type I is  
159 defined by a linear relation between prey density and the number of prey consumed, with a constant intake  
160 rate; type II occurs when the number of prey consumed declines with an increasing prey density until satiation  
161 and is influenced by the time taken to locate, capture and process food (i.e. search coefficient and handling  
162 time); type III is defined by a sigmoidal relation whereby the consumption rate accelerates at low prey  
163 densities, then decreases at higher densities eventually reaching an asymptote (Holling, 1959; Dawes & Souza,  
164 2013). Spiders typically exhibit a type II functional response (Smith, 1984; Řezáč et al., 2010), however, there  
165 are some reported instances of spiders exhibiting a type I and a type III response (Jeschke et al., 2003; Butt &  
166 Xaaceph, 2015; Benhadi-Marín et al., 2019). Observing the functional response of spiders exposed to  
167 insecticides can help determine their potential implications on their function as natural enemies. Although it is  
168 known that insecticides can negatively affect the predatory efficacy of numerous spider species (Pekár, 2012;  
169 Michalko & Košulič, 2016; Butt et al., 2019), there have been few that have investigated how insecticides  
170 affect the predatory behaviours of orb-weavers specifically, despite their evident significance in  
171 agroecosystems (Smith, 1984; Uetz et al., 1999; Řezáč et al., 2010).

172  
173 This study, therefore, investigated the effects of two non-selective synthetic insecticides and one biopesticide,  
174 compared to a control treatment, on the functional response of *Araneus diadematus* towards a generic prey  
175 resource of fruit flies *Drosophila melanogaster*. The focal species is a common orb-weaving spider found in  
176 various habitats throughout Britain (Foelix, 2011; Bee et al., 2019) as well as much of Europe and North  
177 America (Smith, 1984). *A. diadematus* has been reported as an abundant species in field margins and other  
178 agroecosystem habitats (Sloggett, 2010), feeding on a range of insect species, including important pests from  
179 the families Diptera, Heteroptera, Hymenoptera and Coleoptera (Ludy, 2007; Sloggett, 2010). Moreover, *A.*  
180 *diadematus* is a reliable species that has been used for behavioural studies as it can be easily maintained and  
181 readily produces an orb-web in a laboratory setting (Smith, 1984; Zschokke & Herberstein, 2005). The  
182 abundant nature and compatibility with laboratory-based research made *A. diadematus* an ideal organism for  
183 this study.

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## 186 **Methods**

### 187 **Spider collection and maintenance**

188 Immature *A. diadematus* (body length:  $3.8 \pm 0.6\text{mm}$  [mean  $\pm$  SD]) were hand collected from small shrubs and  
189 trees in a grassland habitat in North Ayrshire, Scotland ( $55^{\circ}38'24.4''\text{N}$ ;  $4^{\circ}42'22.6''\text{W}$ ), from July to September  
190 2021. Individual spiders were placed within plastic vials (3cm length; 1cm diameter) and returned to the  
191 laboratory where species confirmation and measurements were made using a stereomicroscope. Individual  
192 spiders were transferred to a container comprised of a wooden frame (20 x 20 x 5cm) with plastic panels (20  
193 x 20 x 0.1cm) placed on either side. The spiders were then held for a 3-day acclimation, starvation and web  
194 construction period. Thereafter, spiders were kept for a further 3-days during which time any spiders that

195 produced an orb web were used for a feeding trial (see below). Spiders were misted with water daily and were  
196 maintained at  $24 \pm 3^\circ\text{C}$  room temperature and  $60 \pm 5\%$  relative humidity, with a natural photoperiod of  
197 approximately 14:10hrs (L:D).

198

### 199 **Insecticide preparation and exposure**

200 Four treatment groups were used in this study consisting of a tap water control, the two synthetic insecticides,  
201 neonicotinoid thiamethoxam (Axoris® Bug Attack; 1% w/w), and pyrethroid deltamethrin (Provanto®  
202 Ultimate Bug Killer; 1.5% w/w), and the biopesticide neem oil (Garten Glück; 1.8% w/w). All insecticides  
203 were applied at the manufacturers' recommended field dosage for foliar application according to the  
204 instructions provided and the correct dilutions were achieved using tap water. When a spider had spun an orb  
205 web, it was carefully removed from its container without damaging the web and was randomly allocated, and  
206 subsequently exposed, to one of the four treatments following a similar method of tarsal exposure by Řezáč et  
207 al. (2010). A piece of filter paper (11cm diameter) was submerged in the respective solution for 5-seconds and  
208 then dried at room temperature for 30-minutes. The filter paper was then rolled and placed into a test tube  
209 (9cm length; 2cm diameter), followed by a spider, and the test tube was plugged with cotton wool to prevent  
210 escape. Spiders were exposed to the treatment for 30-minutes and afterward were removed from the test tube  
211 and placed onto their web once the allocated density of prey had been added (see below). The method of tarsal  
212 exposure could be considered a limitation of the present study as orb-weaving spiders are more likely to  
213 encounter pesticide residue via their webs (Rhoades & Stoddard, 2021), however, this method is well  
214 established and has been demonstrated to be an acceptable form of exposure (e.g. Michalko & Košulič, 2016;  
215 Petcharad et al., 2018; Řezáč et al., 2019a)

216

### 217 **Functional response trials**

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

226

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

233

### 234 **Statistical analysis**

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

240

241 Functional responses were first assessed via logistic regression to determine the shape of the response (i.e.  
242 type I, II or III) by analysing the proportional prey consumption as a function of initial prey density. Here a  
243 significantly negative first-order term indicates a type II response whereas a significantly positive first-order

244 term followed by a significantly negative second-order term indicates a type III response (Juliano, 2001). Once  
245 the general form of the response had been determined, models were fit using a flexible model that induces a  
246 scaling exponent  $q$  to allow for a continuum of shapes between type I, II and III to be described (Barrios-  
247 O'Neill et al., 2015):

$$248 \\ 249 \quad N_e = N_0 (1 - \exp(-b N_0^q (h N_e - T)))$$

250  
251 Where  $N_e$  is the number of prey eaten,  $N_0$  is the initial prey density,  $b$  is the search coefficient,  $h$  is the handling  
252 time,  $q$  is the scaling exponent and  $T$  is the total time available. Where type II responses occur,  $q = 0$ , and  
253 functional responses become increasingly type III in form when  $q > 0$ . In order to compare functional responses  
254 of spiders from different insecticide treatments, 95% confidence intervals were fitted around functional  
255 response curves by non-parametrically bootstrapping the datasets ( $n = 2000$ ). As this allows the functional  
256 response to be considered at the population-level as opposed to the sample-level, differences in functional  
257 responses were made on the basis of confidence interval divergence across prey densities (Pritchard et al.,  
258 2017).

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## 261 **Results**

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

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275

## 276 **Discussion**

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

285

286 This study found that the pyrethroid deltamethrin had the most notable effect on *A. diadematus*. The negative  
287 effects of pyrethroids in spiders have been well documented (Pekár, 2012) where they are known to cause  
288 significant mortalities as well as impair locomotion at sublethal concentrations (Ware & Whitacre, 2004;  
289 Pekár, 2012). Like all synthetic pyrethroids, deltamethrin affects the nervous systems of invertebrates by  
290 holding open the voltage-gated sodium channels found in neuron membranes (Ware & Whitacre, 2004; Garcia,  
291 2011). As a result of the constant excitation of the neuron, deltamethrin causes paralysis and death at lethal  
292 concentrations (Ware & Whitacre, 2004; Garcia, 2011). Paralysis can also occur at sublethal doses, for shorter

293 durations, as well as impaired locomotion, uncoordinated mobility, and convulsions (Garcia, 2011). For  
294 example, a decrease in walking speed when exposed topically to deltamethrin has been observed in the erigonid  
295 spider *Oedothorax apicatus* (Everts et al., 1991) and exposure to  $\lambda$ -cyhalothrin left only the first two pair of  
296 forelegs functional in *Philodromus* spiders (Michalko & Košulič, 2016). In spiders, locomotion is key for all  
297 aspects of foraging and associated predatory behaviours (Chmiel et al., 2000), including the initial construction  
298 of the orb-web and the detection, attack, and capture of prey (Chmiel et al., 2000; Pekár, 2012). Such impacts  
299 have been demonstrated in the orb-weaving spider *Larinioides sclopetarius* whereby the web-building  
300 behaviour and web geometry were negatively affected after exposure to deltamethrin (Lengwiler & Benz,  
301 1994). Similar effects have been reported by other researchers that likewise inferred pyrethroid exposure  
302 impairs coordination of the legs, implicating the locomotion necessary for web construction (Samu & Vollrath,  
303 1992) and predation (Benamú et al., 2013). Although the web structure, and associated web-building  
304 behaviours, were not directly observed in this study, it is plausible that the orb-web suffered adverse alterations  
305 or was perhaps left unattended when damaged after the spiders were exposed to the pyrethroid deltamethrin  
306 (i.e. Samu & Vollrath, 1992; Benamú et al., 2010; 2013). Such effects on the web structure could have further  
307 compromised the predatory efficacy of *A. diadematus* (Benamú et al., 2013; Tew et al., 2015), however, this  
308 cannot be said with certainty. In this study, *A. diadematus* also displayed increased prey handling times and  
309 consumed less prey overall compared to the other treatments, which is likely a result of the impaired mobility  
310 and capture speed due to deltamethrin exposure. It is also possible that paralytic effects of the pyrethroid  
311 impaired function of the chelicera and other mouthparts, preventing *A. diadematus* from efficient consumption  
312 of prey and increasing the handling times, an observation that has been made in other studies (Řezáč et al.,  
313 2010; Michalko & Košulič, 2016).

314  
315 A noteworthy result from the deltamethrin exposed spiders was the shift to a type III functional response.  
316 This is a finding that has been observed previously by Heidari et al. (2015) in the parasitoid *Encarsia*  
317 *formosa* when exposed to the pyrethroid fenpropathrin, implicated by nervous system disruption. Although  
318 information concerning the neurological effects of pyrethroids on spiders is limited (Benamú et al., 2010), it  
319 is probable that predator-prey interactions in this study were also disrupted as a result of an impaired nervous  
320 system (Benamú et al., 2010; Tahir et al., 2019). For example, the prolonged nerve stimulation caused by  
321 deltamethrin exposure has been reported to distort kairomone detection and increase the arrestment response  
322 upon detection, as documented by Delpuech et al. (2005) when studying the effects of deltamethrin on the  
323 parasitoid wasp *Leptopilina heterotoma*. The increased time for a response or reaction upon detecting prey  
324 could, therefore, implicate the search coefficient of *A. diadematus* resulting in the observed lower  
325 consumption rates (Figure 2c). Other pyrethroids have also been known to interfere with sensory receptors  
326 and detection of prey in spiders via kairomones which can reduce the consumption rates in some species  
327 (Desneux et al., 2007). Orb-weavers are sit-and-wait predators that rely on vibratory signals generated by  
328 prey caught in their webs to initiate their capture and immobilisation (Klärner & Barth, 1982; Masters et al.,  
329 1986; Hesselberg, 2015). Therefore, deltamethrin could have potentially interfered with other sensory  
330 receptors in *A. diadematus*, such as those necessary for vibratory detection and olfaction. If the vibratory and  
331 olfactory senses necessary for the detection of prey were distorted in *A. diadematus* then they may not  
332 recognise the prey (Petcharad et al., 2018; Tahir et al., 2019). This could be especially important when  
333 detection of prey at low densities is reduced resulting in a lower consumption rate, and potentially a type III  
334 functional response. In addition, exposure to deltamethrin may have decreased the probability of interaction  
335 between predators and prey because of induced paralysis and impaired mobility in the spiders. Therefore,  
336 even if prey detection by *A. diadematus* was unaffected, the escape rate of prey from the web is still likely to  
337 have been greater than fully functional spiders. A similar consequence has been observed in windy  
338 conditions that impact locomotion and slow the capture speeds of orb-weaving spiders and also lead to an  
339 increased rate of prey escape from the webs (Turner et al., 2011). In our study, there was a shift to a type III  
340 response as a result of deltamethrin exposure, but further research investigating all aspects of predatory  
341 behaviour by *A. diadematus* exposed to this insecticide is required.



342  
343 The other synthetic insecticide in this study, thiamethoxam, surprisingly did not invoke a change in the  
344 functional response of *A. diadematus* compared to the control, and a type II response was observed.  
345 Neonicotinoids, likewise, act on the nervous system, although they block the postsynaptic nicotinic  
346 acetylcholine receptors (Maienfisch, et al., 2001; Ware & Whitacre, 2004; Tomizawa & Casida, 2005). These  
347 receptors bind with the enzyme acetylcholinesterase, which is essential for removing the neurotransmitter  
348 acetylcholine from the synapse (Ware & Whitacre, 2004; Ghosal, 2018). This results in overstimulation of the  
349 neuron, causing uncontrolled and uncoordinated movement, tremors and eventually death at high  
350 concentrations (Ware & Whitacre, 2004). Now, although mortalities have been previously reported in  
351 Lycosidae and Oxyopidae spider populations in response to thiamethoxam (Nazli & Butt, 2020; Lacava et al.,  
352 2021), spiders are generally considered less susceptible to neonicotinoids (Song et al., 2009), which aligns  
353 with our findings. Notwithstanding, studies have documented reduced consumption rates in thiamethoxam  
354 exposed spiders along with impacts to locomotory behaviours, which again has implications for predation  
355 (Butt et al., 2019; Korenko et al., 2019; Řezáč et al., 2021). There is also evidence of species-specific effects  
356 in terms of how exposure occurs and Řezáč et al. (2019a) notably reported that the mode of uptake produced  
357 different results when studying the effects of various neonicotinoids in *Oedothorax apicatus* and *Phylloneta*  
358 *impressa* spiders. Here, dorsal applications resulted in more mortalities than tarsal contact, and when *P.*  
359 *impressa* was exposed to lower concentrations through tarsal contact there were no recorded mortalities (Řezáč  
360 et al., 2019a). In a similar study, the neonicotinoid imidacloprid significantly reduced the predation rate of  
361 *Philodromus cespitum* only through dorsal contact, whereas tarsal exposure had no significant effect (Řezáč  
362 et al., 2019b). Korenko et al. (2019) likewise noted a decrease in prey consumption when *Pardosa agrestis*  
363 were provided with neonicotinoid-treated flies. The form of exposure is evidently an important determinant  
364 for the effects of neonicotinoids in spiders and it is apparent that tarsal exposure is the least detrimental, as  
365 opposed to dorsal application or direct ingestion. In this study, tarsal contact was also the selected method of  
366 exposure, which could potentially explain the negligible effects of thiamethoxam on *A. diadematus*. In this  
367 respect, it is important to consider that orb-weaving spiders are more likely to encounter insecticide residue  
368 through their webs and direct ingestion (Pekár, 1999; Rhoades & Stoddard, 2021), however, in the field orb-  
369 weaving spiders could still uptake insecticide residue through tarsal contact, either from the droplets collected  
370 on the orb-web, or residues on the surrounding substrate (i.e. leaves or branches). Moreover, studies have  
371 demonstrated that some pesticides repel spiders (Pekár & Haddad, 2005; Pekár & Beneš, 2008) and cause them  
372 to abandon their webs (Samu & Vollrath, 1992). This could ultimately increase the likelihood of migrations  
373 across contaminated substrata and thus, the incidence of pesticide exposure via tarsal contact. Future studies  
374 should, however, aim to implement such methods of exposure that closely relate to field conditions.

375  
376 The neem oil treatment also had no effect on the functional response of *A. diadematus* compared to the control  
377 with spiders exhibiting a type II response with similar search coefficients and handling times. Neem-based  
378 products are readily available to control a range of pest species at varying life stages and are therefore a  
379 valuable insecticide for agriculture (Campos et al., 2016; Adhikari et al., 2020). Neem oil, in particular, is the  
380 most widely used neem-based product and it has many pesticidal functions due to a complex composition of  
381 over 200 biologically active compounds facilitating the anti-oviposition, anti-feed and repellent properties  
382 (Stark, 2013; Campos et al., 2016; Gupta et al., 2017; Adhikari et al., 2020). Many studies have determined  
383 that neem oil, along with other selective and botanical insecticides, should have a negligible effect on spiders  
384 and other non-target arthropods in an agroecosystem (Mansour & Nentwig, 1988; Stark, 2013). There is  
385 evidence however of an effect on behaviours in certain species, such as reduced consumption rates in the crab  
386 spider *Philodromus cespitum* (Řezáč et al., 2010). The results from the present study correspond with research  
387 indicating that neem-based products have very minimal sublethal effect on spiders (Mansour & Nentwig, 1988;  
388 Stark, 2013), and are likely safer for the environment than non-selective, synthetic insecticides such as  
389 deltamethrin (Chowański et al., 2014; Haddi et al., 2020). More studies evaluating potential behavioural  
390 impacts of predation in spiders are imperative for the complementary use of neem in agroecosystems.

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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.

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

<b>Treatment</b>	<b>Intercept (p-value)</b>	<b>1<sup>st</sup> order (p-value)</b>	<b>2<sup>nd</sup> order (p-value)</b>	<b>FR type</b>
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

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

<b>Treatment</b>	<b>b±SE (p-value)</b>	<b>h±SE (p-value)</b>	<b>q±SE (p-value)</b>
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

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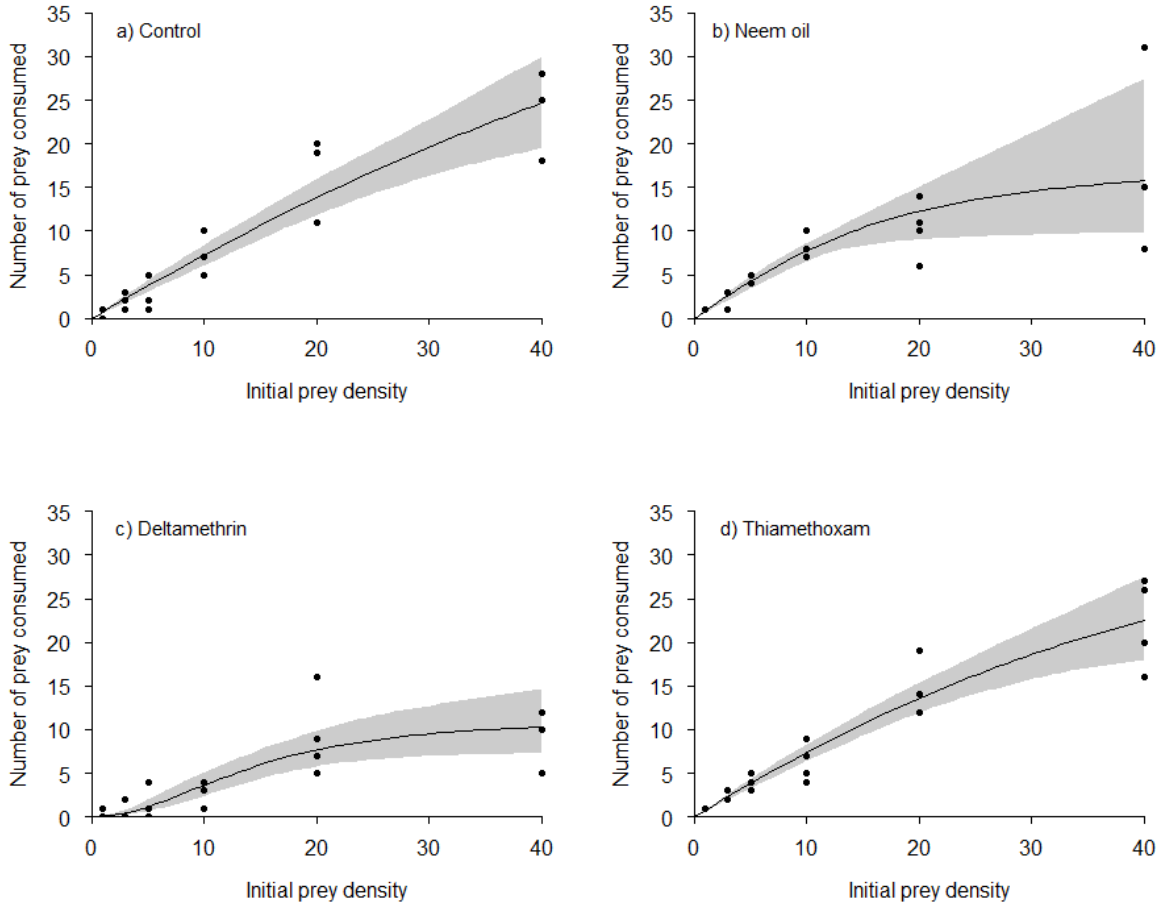
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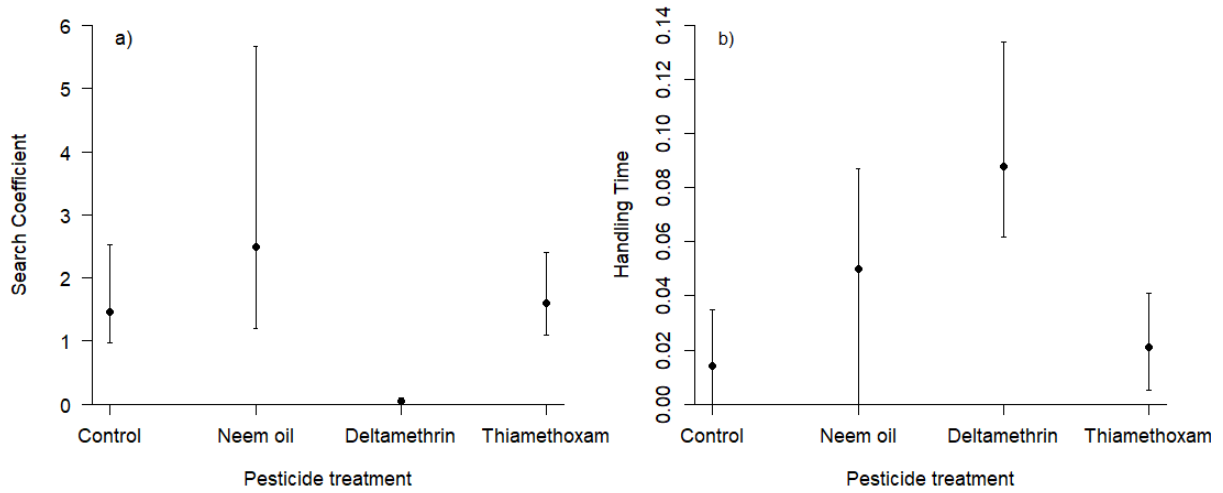


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 759 Figure 1. Functional responses of *A. diadematus* towards *D. melanogaster* when exposed to a)  
 760 control, b) neem oil, c) deltamethrin, and d) thiamethoxam. Solid lines represent the fitted model  
 761 curve and shaded areas represent 95%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

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 767 Figure 2. Functional response model parameters for the a) search coefficient and b) handling time of  
 768 *A. diadematus* when exposed to the control, neem oil, deltamethrin and thiamethoxam treatments  
 769 over a 16-hour period. Solid dots represent the mean values, and the error bars are presented with  
 770 95% confidence intervals calculated by non-parametric bootstrapping (n=2000).  
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