Spider mites collectively avoid plants with cadmium irrespective of their frequency or the presence of competitors

Diogo Prino Godinho*, Inês Fragata* Maud Charlery de la Masselière & Sara Magalhães

cE3c, Centre for Ecology, Evolution and Environmental changes, Faculdade de Ciências da Universidade de Lisboa, Edifício C2, Campo Grande 1749- 016 Lisbon, Portugal.

*co-first authors

Highlights

- Alone, spider mites do not choose between tomato plants with or without cadmium.
- In groups, mites aggregate on plants without cadmium, avoiding contaminated plants.
- Collective avoidance occurs irrespective of the frequency of contaminated plants.
- I The presence of competitors does not affect distribution among cadmium-free plants.
- Spider mites avoid plants with cadmium even when non-contaminated plants have competitors.

Abstract

Plants can accumulate heavy metals from polluted soils on their shoots and use this to defend against herbivory. One possible strategy for herbivores to cope with the reduction in performance imposed by heavy metal accumulation in plants is avoidance of contaminated tissues. Such avoidance, however, may hinge upon the specific conditions faced by herbivores. Here, we tested whether the spider-mite *Tetranychus urticae* avoids tomato

plants contaminated with cadmium in presence of conspecifics or heterospecifics and depending on the frequency of contaminated plants. We show that individual spider mite females do not discriminate between discs with or without cadmium. However, in a set-up where 200 mites were simultaneously given the choice between four plants with or without cadmium, they collectively avoided plants with cadmium. This happened when 50% of the plants were uncontaminated, but also when only a single plant contained no cadmium. In addition, *T. urticae* did not discriminate between plants infested with its competitor *T. evansi* and other non-contaminated plants but they preferred plants with competitors when the other plants contained cadmium. Our results show that aggregation may be an important mechanism through which spider mites avoid contaminated plants. They also indicate that cadmium accumulation in plants is a stronger selective pressure than interspecific competition with *T. evansi.* This suggests that non-accumulating plants will suffer more from herbivory than non-accumulating plants in metal polluted environments. Overall, we show that collective avoidance of contaminated plants by herbivores is robust to environmental conditions and may have important consequences for species distribution and interactions in metal contaminated sites.

Keywords: metal hyperaccumulation; elemental defences; herbivore performance; host choice; interspecific competition.

Introduction

Soils often contain heavy metals such as arsenic, lead, mercurium, chronium and cadmium, either due to geochemical properties or to human activity (Alloway, 2013; Hou et al., 2017). These metals can be taken up by plants, with important consequences for soil quality but also for ecosystem and human health (Cunningham et al., 1995; Gall et al., 2015; Zwolak et al., 2019). Some plants, known as hyperaccumulators, can store metals translocated from the soil in their upper parts, up to levels that are toxic to most organisms (Baker, 1987). Such hyperaccumulation often results in a significant reduction in the performance of herbivores that feed on those plants (Boyd and Moar, 1999; Freeman et al., 2007; Kazemi-Dinan et al., 2014; Mohiley et al., 2020; Quinn et al., 2010). This observation is at the basis of the elemental defence hypothesis, which posits that plants use such hyperaccumulated metals as a defence against herbivores (Martens and Boyd, 1994).

Because metal accumulation is detrimental to most herbivores, one would expect the latter to have evolved means to overcome this effect. Indeed, although elemental defences generally result in a reduction of herbivory, some herbivores may cope with such defences by evolving tolerance mechanisms. Evidence for such adaptation is, however, still scarce. Only a few studies show that herbivore populations have developed mechanisms to metabolize the accumulated toxic compounds (Freeman et al., 2006; Xu et al., 2020), whereas another study shows no adaptation to such selection pressure (Godinho et al., 2023a).

Alternatively (or in addition), herbivores may avoid plants that have accumulated metals. This seems to be a common strategy across species (Kazemi-Dinan et al., 2014; Mogren and Trumble, 2010 but see Vickerman et al., 2003). For example, when given the choice between *Thlaspi caerulascens* plants with high or low zinc concentrations, the desert

locust, *Schistocerca gregaria* preferred the latter, a preference pattern that was recapitulated on artificial food supplemented with zinc (Behmer et al., 2005). Another study showed that cadmium modifies the volatiles of *Populus yunnanensis*, and herbivores (the sawfly *Stauronematus compressicornis* and the leaf beetle *Plagiodera versicolora*) use these cues to avoid those plants (Lin et al., 2022).

Herbivores that have been exposed to contaminated food may use this information to ameliorate their choices. Indeed, grass miners (*Chromatomyia milii*) that have fed on grasses (*Holcus lanatus*) with cadmium avoid these plants more than naïve individuals (Pollard and Baker, 1997). However, feeding on metal-contaminated plants may also impair the behavioural decisions of herbivores (Mogren and Trumble, 2010). This, in turn, may affect their ability to avoid contaminated food. Indeed, the ability of flies (*Drosophila melanogaster*) to avoid substrates with lead vanishes when they have spent their developmental period feeding on lead-contaminated food (Peterson et al., 2020).

Apart from affecting the choice between contaminated and non-contaminated food, exposure to heavy metals may also affect other relevant behavioural decisions in herbivores (Oliveira et al., 2022). For example, in presence of competitors, pyramid ants that had ingested selenium had impaired foraging behaviour (De La Riva and Trumble, 2016). Similarly, exposure to zinc compromised the foraging behaviour and predator avoidance in damselfly larvae (Janssens et al., 2014). Because herbivores are often simultaneously exposed to abiotic and biotic pressures, it is key to understand how their interactive effect on herbivore decisions affect their distribution (e.g. Tran et al., 2019).

Herbivores are rarely alone. The presence of conspecifics may affect their distribution by means of aggregative behaviour (Hunter, 2000; McLellan and Montgomery, 2023). For example, spider mites collectively produce silk balls that they use for dispersal

93 (Clotuche et al., 2011). Herbivores may benefit from being with conspecifics, for example to
94 better overcome plant defences (Wertheim et al., 2005). Moreover, behavioural decisions
95 may be affected by the presence of others (Prokopy and Roitberg, 2001). For example, fruit
96 flies (*Bactrocera tryoni*) avoid low-quality food patches more efficiently when in groups.
97 Knowledge that metals affect collective behaviour has thus far only been gathered in
98 zebrafish (Shelton et al., 2023). Whether organisms collectively avoid metal-contaminated
99 food is expected to hinge upon the relative role of acquisition of information and behavioural
100 impairment that such environments represent.
101 In this study we test whether herbivores avoid plants with cadmium, and whether
102 such avoidance hinges on the presence of conspecifics or heterospecifics, or on the frequency
103 of hyperaccumulating hosts. To this aim, we use the system composed of the spider mites
104 *Tetranychus urticae* and *T. evansi* feeding on tomato plants (*Solanum lycopersicum*). Tomato
105 plants are known to hyperaccumulate cadmium in their upper parts, resulting in decreased
106 performance of the spider mites at high concentrations (Godinho et al., 2022, 2018). *T. urticae*
107 relies on several environmental cues to avoid predators (Pallini et al., 1999), pathogens (Zélé
108 et al., 2019 but see Rodrigues et al., 2022), conspecific (Pallini et al., 1997) and heterospecific
109 competitors (Godinho et al., 2020a). However, whether they avoid metal-accumulating
110 tomato plants and, if so, whether this hinges upon the environmental context, remains an
111 open question.
112

113 **Material and Methods**

114 ***Plant and mite rearing conditions***

115 Tomato plants (*Solanum lycopersicum*, *var* MoneyMaker) were sown in a climatic room (25ºC,
116 61% of humidity, light:dark = 16:8), in pots with soil (pH 5.0-6.0; SIRO, Portugal) and watered

117 twice a week with 100 mL of tap water for the two first weeks. Subsequently, plants were
118 transplanted to a mixture of 1:4 vermiculite, gardening soil (SIRO, Portugal), where they grew
119 for three more weeks before they were used in the experiments. During those weeks, plants
120 were watered twice a week with 100 mL of either distilled water or a cadmium solution
121 (3mM). All plants were also watered once a week with tap water to avoid the lack of nutrients.
122 Just before the installation of the setup, each plant was watered with 70 mL of tap water. This
123 concentration of cadmium ensures an amount accumulated by tomato plants higher than the
124 hyperaccumulating threshold (Godinho et al., 2018) and falls within values encountered in
125 natural populations of hyperaccumulating plants (Meyer et al., 2015).

126 The populations of spider mites used in this study were created in 2018 by performing
127 controlled crosses between three populations of *T. urticae* and four populations of *T. evansi*
128 collected in the field of Portugal in 2017 from tomato plants (Godinho et al., 2020b). The
129 resulting outbred populations have been maintained in high numbers (> 1000 adult females)
130 in cages (28 x 39 x 28 cm) each containing two entire cadmium-free tomato plants, under
131 controlled conditions (25 ºC ± 1, 68% of humidity, light: dark = 16:8) in a climatic room at the
132 University of Lisbon. In the case of individual choice and performance, populations were
133 maintained in 5 replicates during 14 discrete generations with 220 adult mites being
134 transferred every 14 days, as part of a larger experiment. To ensure that females used in the
135 experiments were approximately of the same age, adult females where isolated on separate
136 tomato leaves in plastic boxes (14 x 20 x 14 cm) and allowed to lay eggs for 48h. In all
137 experiments, we used adult females resulting from this cohort, 15 days after egg laying.

138

139 ***Experimental set-up for individual choice and performance***

140 To test whether spider mites individually choose between leaf discs with or without cadmium,
141 mated females were placed at the centre of plastic strips (1x3 cm) connecting two leaf discs
142 (Ø 16 mm) placed on soaked cotton wool, one made from a tomato plant supplied with
143 cadmium (2 mM) and the other from a plant without cadmium (Figure 1A). For each replicate,
144 leaf discs were made from leaves of the same age (either the 3rd or 4th counting from the
145 cotyledons) and the age and position of the disc with cadmium was randomized across trials.
146 Females (N = 17 to 24 per replicate population; total N = 101) were allowed to choose
147 between leaves with or without cadmium for 24h, after which the position of the female was
148 registered. To measure the effects of each environment on the performance of spider mites,
149 females (N = 13 to 20 per replicate population for each environment; N = 94 for plants with
150 no cadmium and N= 97 for plants with cadmium) were isolated on leaf discs (Ø 18 mm) made
151 from leaves 3 to 5 (from the cotyledons), from plants supplied with 2mM cadmium chloride
152 or not. These leaf discs were kept on top of soaked cotton wool in petri dishes. 48 hours later,
153 females were killed, and the number of eggs was counted. Assays were divided in blocks
154 consisting of four consecutive days.
155
156 ***Experimental set-up for collective choice and performance***
157 To measure how the presence of competitors and the frequency of plants with cadmium
158 affected the collective choice of spider mites, four entire tomato plants were placed in a
159 plastic box and connected by a round plastic arena (15 cm of diameter) in which their 4th leaf
160 was inserted in a hole of 2 cm long at equal distance from each other and from the centre of
161 the arena (Fig. 1B). We applied a ring of Vaseline around the petiole of the 4th leaf close to
162 the node to prevent mite dispersal to other leaves of the plant. We then released 200 females
163 of *T. urticae* in the centre of the plastic arena, from where they could move to any plant, and

164 counted the number of mites on each plant 24 hours later. This setup was used to test
165 whether spider mite distribution was random or affected by the choice of conspecifics (i.e., if
166 there was aggregation). This was done by giving spider mites the choice between four
167 uninfested plants non-contaminated with cadmium (treatment 1; Figure 1B). Additionally, we
168 used this set up to address how the distribution of spider mites among plants was affected by
169 the frequency of contaminated hosts and the presence of a competitor in stressful vs non-
170 stressful environments. To address the first question, we placed mites in set-ups with either
171 two (treatment 2) or three (treatment 3) plants with cadmium (Figure 1B), with the remaining
172 plants being cadmium-free. To answer the second question, we created two other
173 treatments, in which the leaf of one plant without cadmium was infested with 100 adult
174 females of the competitor species *T. evansi* (50 females on the first leaflet and 25 on the two
175 lateral leaflets to avoid overexploitation by the competitor). This plant was surrounded by
176 three plants either with (treatment 4) or without cadmium (treatment 5; Fig. 1B). The 200 *T.*
177 *urticae* females were placed in the centre of the plastic arena 24 hours after the installation
178 of *T. evansi*. The number of *T. urticae* females on each plant was counted 24 hours later.

179 To measure the performance of *T. urticae* on plants with or without cadmium, the
180 number of eggs on each leaf was counted on all plants in treatments 1, 2 and 3 after having
181 counted (and removed) the females to measure choice. The number of eggs was then divided
182 by the number of females on the same plant, such as to obtain the per capita oviposition rate.
183 To measure the performance of *T. urticae* in presence of the competitor (treatments 4 and
184 5), we quantified the number of adult female offspring after fifteen days, because the eggs of
185 the two species are not distinguishable. To this aim, we removed the adult females from the
186 infested leaf of the plant with competitors (from treatments 4 and 5) and the infested leaf of
187 one random plant without cadmium in the respective controls (treatments 1 and 3). Each leaf

188 was detached from the plant and placed in a pot with water in an isolated box and fifteen
189 days later, the number of adult females was counted. Twenty replicates per treatment were
190 done (except for treatment 5 for which 19 replicates were done).
191
192 ***Statistical analysis***
193 Individual choice and performance
194 To test if individual females choose between cadmium or cadmium-free leaf discs, a general
195 linear mixed model with a Binomial error distribution was used. The dependent variable was
196 the presence or absence (coded as 1 or 0, respectively) of the female in each environment
197 (using the cbind function in R), and leaf age and replicate were used as random factors. To
198 test the effect of cadmium on the female performance, the number of eggs laid on each patch
199 was analysed using a general linear model with a Poisson error distribution. The dependent
200 variable was the number of eggs per patch, the test environment (cadmium-free vs. cadmium
201 leaf discs) was coded as a fixed factor and leaf age and replicate were used as random factors.
202 Collective choice and performance
203 We first tested whether spider mites distribute themselves evenly across cadmium-free
204 plants. To this aim, we performed a G-test goodness of fit per replicate, assuming that the
205 mites would distribute equally across plants (*i.e.*, 25% per plant). To control for multiple tests,
206 a Bonferroni correction was applied. In addition, we performed a general linear model
207 comparing the control treatment to a mock treatment where each plant 'received' 50
208 individuals of the 200 released.
209 All statistical analyses to test the collective spider mite behaviour were done using a
210 general linear model with number of individuals on a plant as the dependent variable. All

models included block and interaction between identity of the plant and replicate as random factors. Models where initially fit with a Poisson distribution, but due to overdispersion, a negative binomial error distribution was used instead.

To test whether spider mites collectively avoided plants with cadmium, we compared the distribution of females in plants with and without cadmium (treatment 2, 3 and 4) using a model with cadmium and treatment as fixed factors and their interaction. Post hoc contrasts were performed to test whether spider mites avoided plants with cadmium in each treatment, and to test whether treatments differed concerning this avoidance. To test if cadmium concentration affected spider mite choice we compared treatments 2 and 3, and to test if presence of competitors affected choice of plants with cadmium we compared treatments 3 and 4. Finally, we tested whether the presence of cadmium in the environment affected the avoidance of competitors by comparing the treatments containing a plant with competitors (treatments 4 and 5) with a model with competitors and treatment as fixed factors and their interaction. Post hoc contrasts were performed to test whether spider mites avoided plants with competitors in each treatment, and whether treatments differed concerning this avoidance.

All statistical analyses to test collective spider mite performance were done using general linear models with a gamma error distribution, and the number of per capita eggs or the number of per capita female offspring (for the treatments with competitors) as dependent variables. All models included block and replicate as random factors.

We first assessed spider mite performance in the control treatment (i.e., treatment 1) to test whether mite oviposition rate was even across plants. This was done using a generalized mixed model with plant as fixed factor. The effect of cadmium on spider mite

234 performance was assessed by comparing the per capita oviposition rate across all plants
235 among treatments containing plants with and without cadmium (i.e., treatments 2, 3 and 4).
236 For that we applied a model with cadmium and treatment and their interaction as fixed
237 factors. Next, we performed pairwise comparisons to test whether the performance of spider
238 mites differed on plants with or without cadmium within each treatment. We also performed
239 pairwise comparisons to test whether the performance on plants with cadmium differed
240 across treatments, only using data from plants with cadmium in each treatment.

241 To estimate if the effect of cadmium was similar for the individual and collective tests,
242 we calculated the standardized effect size for each treatment and experiment using the
243 effectsize package (Ben-Shachar et al., 2020).

244 To test the effect of the competitor on the number of per capita offspring we used a
245 model with presence (or absence) of competitors as fixed factor. We used posthoc tests to
246 compare the treatments with competitors and their respective control (i.e. treatment 1 vs
247 treatment 5 and treatment 3 vs treatment 4).

248 All statistical analyses were performed using the software R v3.6 (R Core Team, 2022),
249 using packages glmmTMB and lme4 to perform the general linear models (Bates et al., 2015;
250 Brooks et al., 2017), car for the ANOVA tests (Fox and Weisberg, 2019), emmeans for post-
251 hoc contrasts (Lenth, 2023) and ggplot2 for visualization (Wickham, 2009).

252

253 **Results**

254 Individual choice and performance

255 Individual spider mite females did not occupy differently leaf discs with and without cadmium
256 (estimated overall probability = 0.0554, z ratio= 1.064, P = 0.2875, Fig. 2A). They laid

257 significantly fewer eggs on leaf discs with cadmium than on leaf discs without cadmium (ratio
258 between water and cadmium= 2.1, z ratio= 10.432, p<0.001, Fig. 2B, Table S1).
259
260 Collective choice and performance
261 Spider mites distributed themselves unevenly across plants in our set-up. In 18 out of the 19
262 replicates, they showed a distribution significantly different from the expected 25%, even
263 after Bonferroni correction (Table S2). Indeed, the difference between the treatment with
264 only plants with no cadmium and no competitors and a mock variable with an even
265 distribution across plants was significant (effect of treatment: X^2_2=7410,5; P < 0.0001, Fig 3,
266 treatment 1).
267 There was a significant effect of cadmium on the number of females on each plant,
268 but not of treatment or their interaction (ANOVA results cadmium: X^2_1 =8.70, p-value
269 =0.0031; treatment: X^2_1 = 1.678, p-value= 0.432; cadmium*treatment: X^2_1 = 1.674, p-value =
270 0.432 ; Fig. 3). Spider mites avoided plants with cadmium in all treatments (treatment 2
271 plants: z ratio=-55.987, p-value <0.0001, treatment 3: z ratio=-49.560, p-value <0.0001;
272 treatment 4: z ratio=- 49.343, p-value <0.0001). This avoidance was not different between
273 treatments with two or with three plants (contrast: z ratio=-0.390, p value = 0.9196).
274 There was a significant effect of the presence of a competitor, and the interaction with
275 treatment on the number of females on each plant (competitor: X^2_1 = 4.556, p-value= 0.033;
276 treatment: X^2_1 = 1.666, p-value=0.197; cadmium*treatment: X^2_1 = 5.556, p-value =0.0184; Fig.
277 3). There was no difference in the number of mites present in plants with or without
278 competitors when all plants were cadmium-free (contrast: z ratio=1.203, p-value = 0.229).
279 However, in the treatment where plants that were not infested with the competitor had
280 cadmium, females chose more often the plant with the competitor (i.e. avoided cadmium),

281 resulting in a significant difference among these treatments (contrast: z ratio= -2.134, p-value
282 = 0.033). This choice was not different from that documented when the non-contaminated
283 plant did not have competitors (contrast between treatment 3 and 4: z ratio=-0.457, p value=
284 0.891).

285 In the control treatment, plant identity did not significantly affect spider mite
286 oviposition rate (X^2_3 = 3.473, p-value = 0.324; Figure 4).

287 The presence of cadmium in plants, in treatments 2, 3 and 4 significantly reduced the
288 oviposition rate of females (X^2_2 = 89.745, p-value <0.0001; Figure 4). However, having two or
289 three plants with cadmium in the setup did not affect spider-mite performance (X^2_2 = 0.413,
290 p-value = 0.813; Figure 4). In fact, the standardized effect size associated with the presence
291 of cadmium was remarkably similar for treatments 2 and 3 (Table S1). The effect size of
292 cadmium is larger in individual than in collective performance experiments, but showing a
293 clear overlap of the confidence intervals (Table S1).

294 In environments with cadmium-free plants only, there was a significant effect of the
295 presence of the competitor on the number of offspring per female after one generation (X^2_1
296 = 15.877, p-value = 0.0004, Figure 5). Contrasts between plants with or without competitors
297 revealed that the number of female offspring on uninfested plants (*i.e.*, in the absence of
298 competitors or cadmium) was higher than on cadmium-free plants with competitors
299 (contrasts between treatment 1 and 5: t ratio= 3.771, p-value=0.002). However, this
300 difference was not observed when competitor-free plants contained cadmium (contrasts
301 between treatment 3 and 4: t ratio= -1.713, p-value=0.325).

302

303 **Discussion**

304 Our study shows that *T. urticae* females do not avoid tomato leaves with cadmium when they
305 are given the choice individually, although feeding on these leaves entails a reduction in their
306 performance. In contrast, when in group, facing the choice between cadmium and cadmium-
307 free plants, females clearly moved more often to plants without cadmium. They also showed
308 a non-even distribution, even when all plants were uninfested and uncontaminated,
309 suggesting aggregation behaviour. Moreover, the frequency of cadmium-free plants had no
310 effect on spider-mite avoidance. Finally, when all plants were cadmium-free spider mites did
311 not discriminate between plants with or without competitors, but they avoided plants with
312 cadmium when the alternative was moving to a non-contaminated plant with competitors.

313 We found here that spider mites feeding tomato plants that have hyperaccumulated
314 cadmium have a lower oviposition rate than mite feeding on non-contaminated plants. This
315 result recapitulates previous findings (Godinho et al., 2023b, 2022, 2018), and implies that
316 they are robust to the set-up used, be it on single individuals on leaf discs or on populations
317 on detached leaves. Curiously, however, when given the choice between leaf discs with or
318 without cadmium, *T. urticae* females did not avoid the former. Although this recapitulates a
319 result obtained for *T. evansi* (Godinho et al., 2023a), it is surprising, not only because of the
320 abovementioned consequences of cadmium for performance, but also because this choice
321 set-up had been validated previously (Rodrigues et al., 2017; Zélé et al., 2019). The
322 abovementioned set-ups included cues from conspecifics or from bacteria, whereas the leaf
323 discs used here differed solely in cues stemming from the plant itself. Hence, possibly,
324 cadmium does not affect plant cues that inform spider mites of its content. Alternatively, such
325 cues may be produced in whole plants but not in cut leaf discs.

326 In contrast to the individual choice assays, spider mites systematically avoided plants with
327 cadmium in a set-up that simultaneously tested 200 mites. Our results from the control

328 treatment unequivocally show that spider mites do not distribute themselves evenly across
329 plants. Instead, they tend to aggregate on particular plants, a result also found in the field
330 (Rijal et al., 2016). Aggregation behaviour has been shown to be beneficial to *T. urticae*, by
331 increasing fecundity and net production and decreasing the death rate of females living in
332 groups (Le Goff et al., 2010). In the treatments containing plants with and without cadmium,
333 such aggregation occurred on plants without cadmium. It is thus tempting to hypothesize that
334 spider mites use the information of their conspecifics to avoid plants with cadmium. Spider
335 mites often communicate by means of odour cues, for example when searching for mates
336 (Regev and Cone, 1976; Rodrigues et al., 2017) or when avoiding predators (Choh and
337 Takabayashi, 2010; Janssen et al., 1999). Possibly, aversive chemical information of spider
338 mites that have been on plants with cadmium is available in our set-up and used by spider
339 mites to make choices that are beneficial for their performance. Alternatively, cadmium
340 disrupts the production of plant volatiles induced by spider mites, which are also used by
341 conspecifics to find infested plants (Lin et al., 2022; Pallini et al., 1997). In this case, cadmium-
342 free plants are expected to become more attractive than plants with cadmium.

343 In environments without cadmium, *T. urticae* spider mites did not avoid plants with
344 competitors when given the choice between such plants and plants without competitors,
345 which is at odds with the number of offspring they produce after one generation being lower
346 on plants with competitors as compared to uninfested plants, as found here and in other
347 studies . (Fragata et al., 2022; Sarmento et al., 2011a). Possibly, avoidance of *T. evansi* to other
348 plants is only observed in later stages of plant infestation, when resources become scarcer.
349 Indeed, *T. evansi* is known to downregulate plant defences, which is beneficial to *T. urticae* in
350 the short term (Godinho et al., 2016; Sarmento et al., 2011b). Moreover, *T. urticae* is able to

351 avoid *T. evansi* within a single plant (Godinho et al., 2020a), which may release the selection
352 pressure to avoid whole plants.

353 In contrast, the presence of cadmium on plants uninfested with the competitor
354 changes the behaviour of *T. urticae* towards this competitor. Our results suggest that spider
355 mites avoid plants with cadmium in detriment of avoiding competitors. An earlier study had
356 shown that spider mites prefer young over old leaves, as the former result in higher
357 oviposition rate for them, but that this preference vanishes when this leaf is infested with
358 competitors (Godinho et al., 2020a). Here, instead, we show that an abiotic selection pressure
359 seems to be more relevant to spider mite host choice than the presence of competitors. The
360 complex interaction between responses to abiotic and biotic stressors has been documented
361 in other systems (Murphy and Loewy, 2015; Tran et al., 2019). One possible explanation for
362 our results is that selecting hosts with *T. evansi* might be less risky than selecting plants with
363 cadmium because *T. urticae* could still avoid *T. evansi* once on the plant by finding a leaf free
364 of competitors (e.g.,Godinho et al., 2020a), while cadmium is accumulated in all leaves and
365 there is no way to avoid it within a plant.

366 The avoidance of cadmium even in the presence of competitors implies stronger
367 competition on cadmium-free plants. This is in line with studies showing increased intra or
368 interspecific competition in resource patches with higher quality in heterogeneous
369 environments (Shen et al., 2020; Wang et al., 2012). This suggests that spider mites will be
370 exposed to different selection pressures in heterogeneous environments: on plants with
371 cadmium, selection will favour mites that are able to physiologically cope with the toxic
372 effects of metal, whereas on plants with no cadmium mites will face stronger intra- and inter-
373 specific competition. If these two selection pressures persist for long enough, they may lead
374 to high population differentiation within herbivores. Corroborating this hypothesis would

375 necessitate studying populations occurring on sites with both types of plants (e.g. Freeman
376 et al., 2007).

377 Alternatively, spider mites will not be able to adapt to plants with cadmium, for
378 example due to lack of genetic diversity, in which case the observed cadmium avoidance
379 would lead to plants with such metal suffering few attacks by herbivores in the long run, as
380 seen in other systems (Galeas et al., 2008; Noret et al., 2007). This should select for the
381 maintenance of metal accumulation in plants, especially because spider mites do not
382 individually avoid tomato plants, and not all mites avoid plants, even when collectively
383 choosing. Thus, plants with cadmium will still suffer some herbivore damage. Hence, as long
384 as the fitness cost of uptaking metal does not surpass the fitness gain of herbivore avoidance,
385 selection to uptake metals as a defence against herbivores will be maintained.

386 In sum, our results support that collective choice may help the maintenance of plant
387 elemental defence against herbivory, by increasing avoidance of contaminated plants, while
388 also revealing that hyperaccumulation by plants may indirectly affect competition among
389 herbivores, thus, having broad effects on metal polluted ecosystems.

390

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397 **Conflict of interest**

398 Authors declare no conflict of interest.

Data availability

Data will be deposited in Figshare upon acceptance of the manuscript.

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608 **Figures:**

609 **Figure 1.** Experimental set-ups to measure individual (A) and collective host choice (B) and
610 performance (C). The white and black shapes represent plants without and with cadmium,
611 respectively. A) Experimental set-up for individual host choice (left panel) and individual
612 performance (right panel). B) Experimental set-up for collective host choice and performance
613 and schematic representation of the 5 treatments. The leaf marked in red streaks (treatments
614 4 and 5) identifies the presence of competitors.

615

616 **Figure 2. Individual host choice and performance**

617 (A) Number of females on leaf discs from plants with cadmium (dark grey) or without
618 cadmium (light grey) after 24 hours. (B) Number of eggs laid on leaf discs from plants with or
619 without cadmium. Error bars represent replicate standard error between replicates.

620

621 **Figure 3. Collective host choice.** Number of females after 24h on each plant for all treatments.
622 Numbers indicate the treatments: 1) control, 2) two plants with cadmium, 3) three plants with
623 cadmium, 4) one plant infested with competitors, 5) one plant infested with competitors and
624 three plants with cadmium. Dark grey bars represent plants with cadmium, light grey colour
625 plants without cadmium or competitor, white bars represent presence of competitors on the
626 plants. Error bars represent replicate standard error between replicates.

627

628 **Figure 4. Collective performance.** A) Number of eggs per female after 24h on each plant for
629 treatments 1-3. Dark grey bars represent plants with cadmium, light grey colour plants
630 without cadmium or competitor, white bars represent presence of competitors on the plants.
631 Error bars represent replicate standard error between replicates.

632 **Figure 5. Collective performance in presence of competitors.** Light grey bars represent plants
633 without cadmium or competitor, white bars represent presence of competitors on the plants.
634 Error bars represent replicate standard error between replicates. Both types of plants
635 contained no cadmium.
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637
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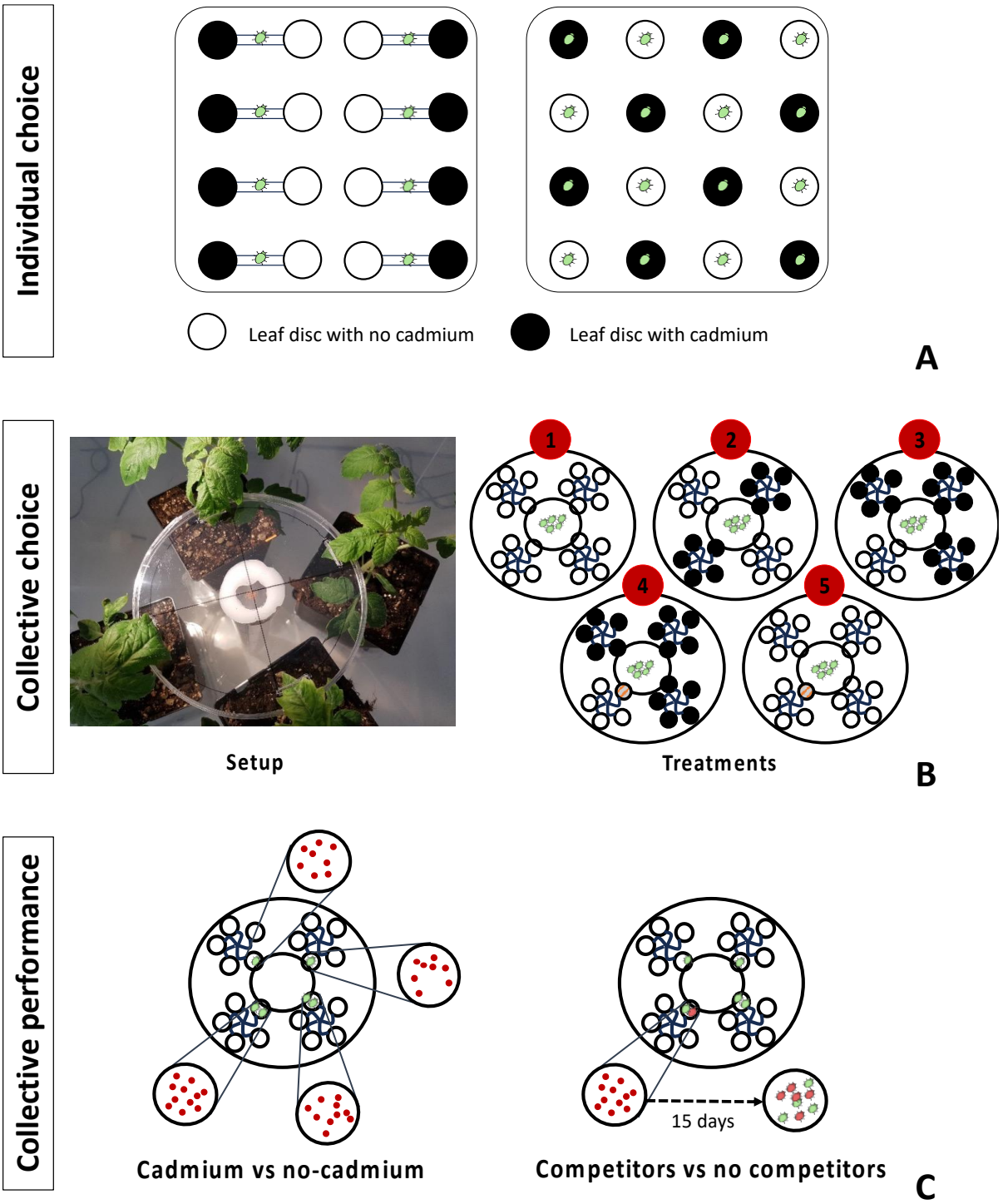
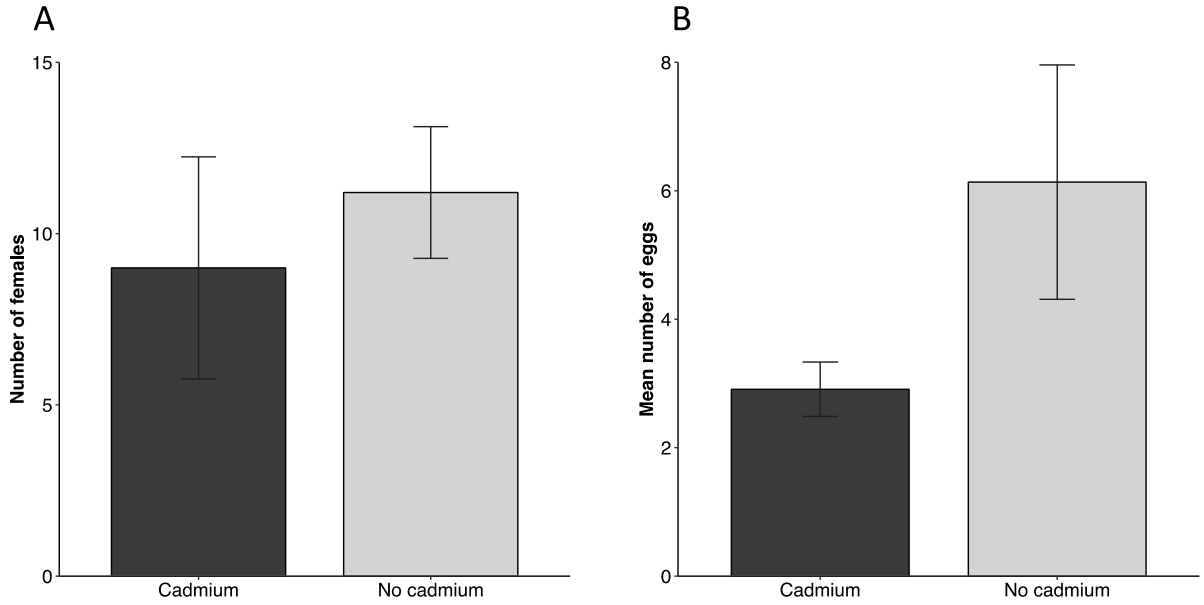
Figure 1


Individual choice
Leaf disc with no cadmium
Leaf disc with cadmium
A
Collective choice
Setup
1
2
3
4
5
Treatments
B
Collective performance
Cadmium vs no-cadmium
15 days
Competitors vs no competitors
C

643 **Figure 2**



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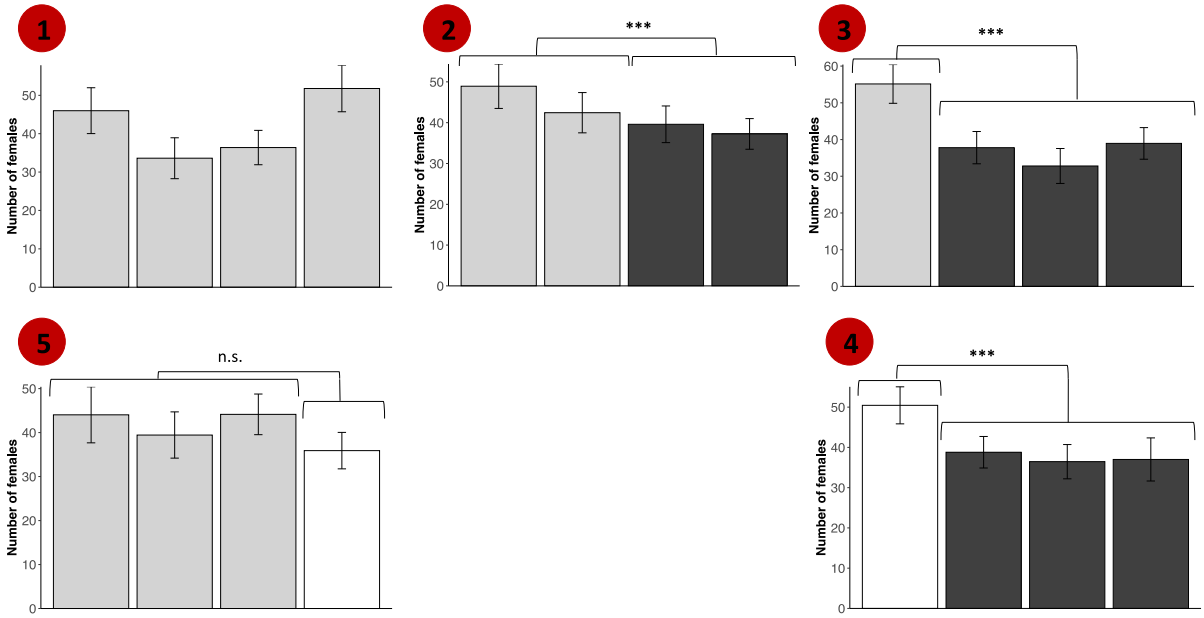
647 **Figure 3**



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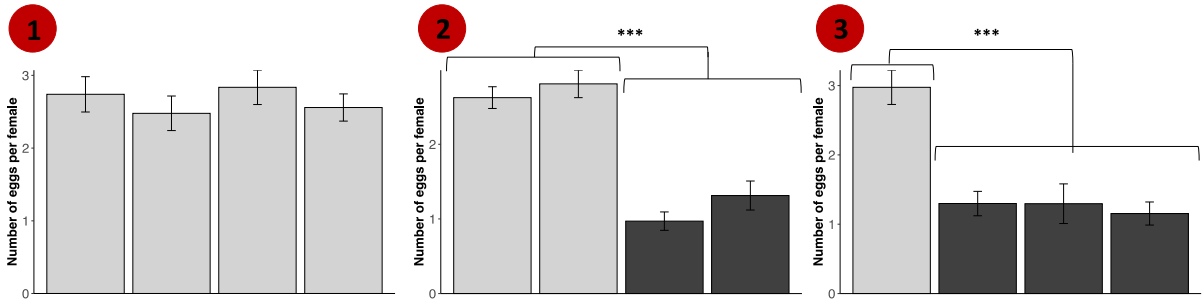
650 **Figure 4**



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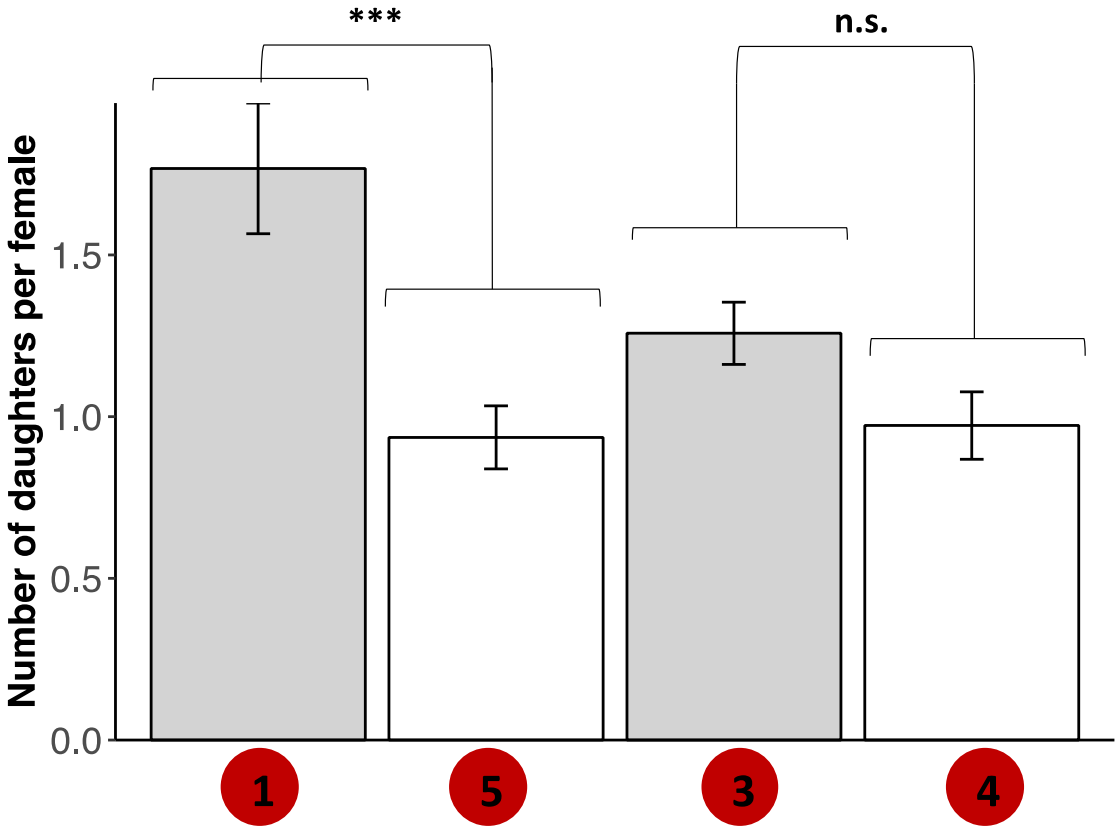
654 **Figure 5**



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