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

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Abstract

1. Plants can accumulate heavy metals from polluted soils on their shoots and use this to defend themselves 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.
2. 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.
3. 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 uncontaminated plants but they preferred plants with competitors when the other plants contained cadmium.

4. 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 accumulating plants in metal polluted environments.

5. *Synthesis and applications*. We show that collective avoidance of metal-accumulating 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, either due to geochemical properties or to human activity (Alloway, 2013; Hou et al., 2017). 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 & 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 & Boyd, 1994).

Because metal accumulation is detrimental to most herbivores, one would expect the latter to evolve means to overcome this effect. Indeed, some herbivores may cope with elemental defences by evolving tolerance mechanisms. Evidence for such adaptation is, however, still scarce(Freeman et al., 2006; Xu et al., 2020), and not universal (Godinho, et al., 2023a).

Alternatively (or in addition), herbivores may avoid plants that have accumulated metals. This seems to be a common strategy (Kazemi-Dinan et al., 2014; Mogren and Trumble, 2010; 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 & Baker, 1997). However, feeding on metal-contaminated plants may also impair the behavioural decisions of herbivores (Mogren & Trumble, 2010). This, in turn, may affect their ability to avoid contaminated

65 food. Indeed, the ability of flies (*Drosophila melanogaster*) to avoid substrates with lead vanishes
66 when they develop on lead-contaminated food (Peterson et al., 2020).

67 Apart from affecting the choice between contaminated and non-contaminated food,
68 exposure to heavy metals may also affect other relevant behavioural decisions in herbivores
69 (Oliveira et al., 2022). For example, in presence of competitors, pyramid ants that had ingested
70 selenium had impaired foraging behaviour (De La Riva & Trumble, 2016). Similarly, exposure to zinc
71 compromised the foraging behaviour and predator avoidance in damselfly larvae (Janssens et al.,
72 2014). Because herbivores are often simultaneously exposed to abiotic and biotic pressures, it is key
73 to understand how their interactive effect on herbivore decisions affect their distribution (Tran et
74 al., 2019).

75 Herbivores are rarely alone. The presence of conspecifics may affect their distribution by
76 means of aggregative behaviour (Hunter, 2000; McLellan & Montgomery, 2023). Herbivores may
77 benefit from being with conspecifics, for example to better overcome plant defences (Wertheim et
78 al., 2005). Moreover, behavioural decisions may be affected by the presence of others (Prokopy &
79 Roitberg, 2001). For example, fruit flies (*Bactrocera tryoni*) avoid low-quality food patches more
80 efficiently when in groups (Morimoto et al., 2018).

81 Knowledge that metals affect collective behaviour has only been gathered in zebrafish
82 (Shelton et al., 2023). Whether organisms collectively avoid metal-contaminated food is expected to
83 hinge upon the relative role of acquisition of information and behavioural impairment that such
84 environments represent.

85 In this study, we test whether herbivores avoid plants with cadmium and whether such
86 avoidance hinges on the presence of conspecifics or heterospecifics, or on the frequency of
87 hyperaccumulating hosts. To this aim, we use the system composed of the spider mites *Tetranychus*
88 *urticae* and *T. evansi* feeding on tomato plants (*Solanum lycopersicum*), which hyperaccumulate
89 cadmium in their upper parts, resulting in decreased performance of the spider mites at high
90 concentrations (Godinho et al., 2018, 2022). *T. urticae* relies on several environmental cues to avoid

91 predators (Pallini et al., 1999), pathogens (Zélé et al., 2019) but see (Rodrigues et al., 2022), conspecific
92 (Pallini et al., 1997) and heterospecific competitors (Godinho, et al., 2020a). However, whether they
93 avoid metal-accumulating tomato plants and, if so, whether this hinges upon the environmental
94 context, remains an open question.
95

96 **Material and Methods**

97 ***Plant and mite rearing conditions***

98 Tomato plants (*Solanum lycopersicum*, *var* MoneyMaker) were sown in a climatic room (25ºC, 61% of
99 humidity, light:dark = 16:8), in pots with soil (pH 5.0-6.0; SIRO, Portugal) and watered twice a week
100 with 100 mL of tap water for the two first weeks. Subsequently, plants were transplanted to a mixture
101 of 1:4 vermiculite, gardening soil (SIRO, Portugal), where they grew for three more weeks and were
102 watered twice a week with 100 mL of either distilled water or a cadmium solution (3mM or 2mM
103 according to the experiment, see below). All plants were also watered once a week with tap water to
104 avoid the lack of nutrients. Just before the installation of the setup, each plant was watered with 70
105 mL of tap water. This concentration of cadmium added to the soil ensures that the amount
106 accumulated by tomato plants in their leaves is higher than the hyperaccumulating threshold
107 (Godinho et al., 2018) and falls within values encountered in natural populations of hyperaccumulating
108 plants (Meyer et al., 2015).

109 The populations of spider mites used in this study were created in 2018 by performing
110 controlled crosses between three populations of *T. urticae* and four populations of *T. evansi* collected
111 in the field of Portugal in 2017 from tomato plants (Godinho et al., 2020b). The resulting outbred
112 populations have been maintained in high numbers (> 1000 adult females) in cages (28 x 39 x 28 cm)
113 each containing two entire cadmium-free tomato plants, under controlled conditions (25 ºC ± 1, 68%
114 of humidity, light: dark = 16:8). For the experiment on individual choice and performance, populations
115 were maintained in 5 replicates during 14 discrete generations with 220 adult mites being transferred
116 every 14 days, as part of a larger experiment. To ensure that females used in the experiments were

117 approximately of the same age, adult females where isolated on separate tomato leaves in plastic
118 boxes (14 x 20 x 14 cm) and allowed to lay eggs for 48h. In all experiments, we used adult females
119 resulting from this cohort, 14 days after egg laying.

120 The animals used in this research are not vertebrates or invertebrates with ethical and care
121 limitations.

122

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

124 To test whether spider mites individually choose between leaf discs with or without cadmium, mated
125 females were placed at the centre of plastic strips (1x3 cm) connecting two leaf discs (Ø 16 mm) placed
126 on soaked cotton wool, one made from a tomato plant supplied with cadmium (2 mM) and the other
127 from a plant without cadmium (Figure 1A). For each replicate, leaf discs were made from leaves of the
128 same age (either the 3rd or 4th counting from the cotyledons) and the age and position of the disc with
129 cadmium was randomized across trials. Females (N = 17 to 24 per replicate population; total N = 101)
130 were allowed to choose between leaves with or without cadmium for 24h, after which the position of
131 the female was registered. To measure the effects of each environment on spider mite performance,
132 females (N = 94 for plants with no cadmium and N= 97 for plants with cadmium) were isolated on leaf
133 discs (Ø 18 mm) made from leaves 3 to 5 (from the cotyledons), from plants supplied with 2mM
134 cadmium chloride or not. These leaf discs were kept on top of soaked cotton wool in petri dishes. 48
135 hours later, females were sacrificed and the number of eggs was counted. Assays were divided in
136 blocks consisting of four consecutive days.

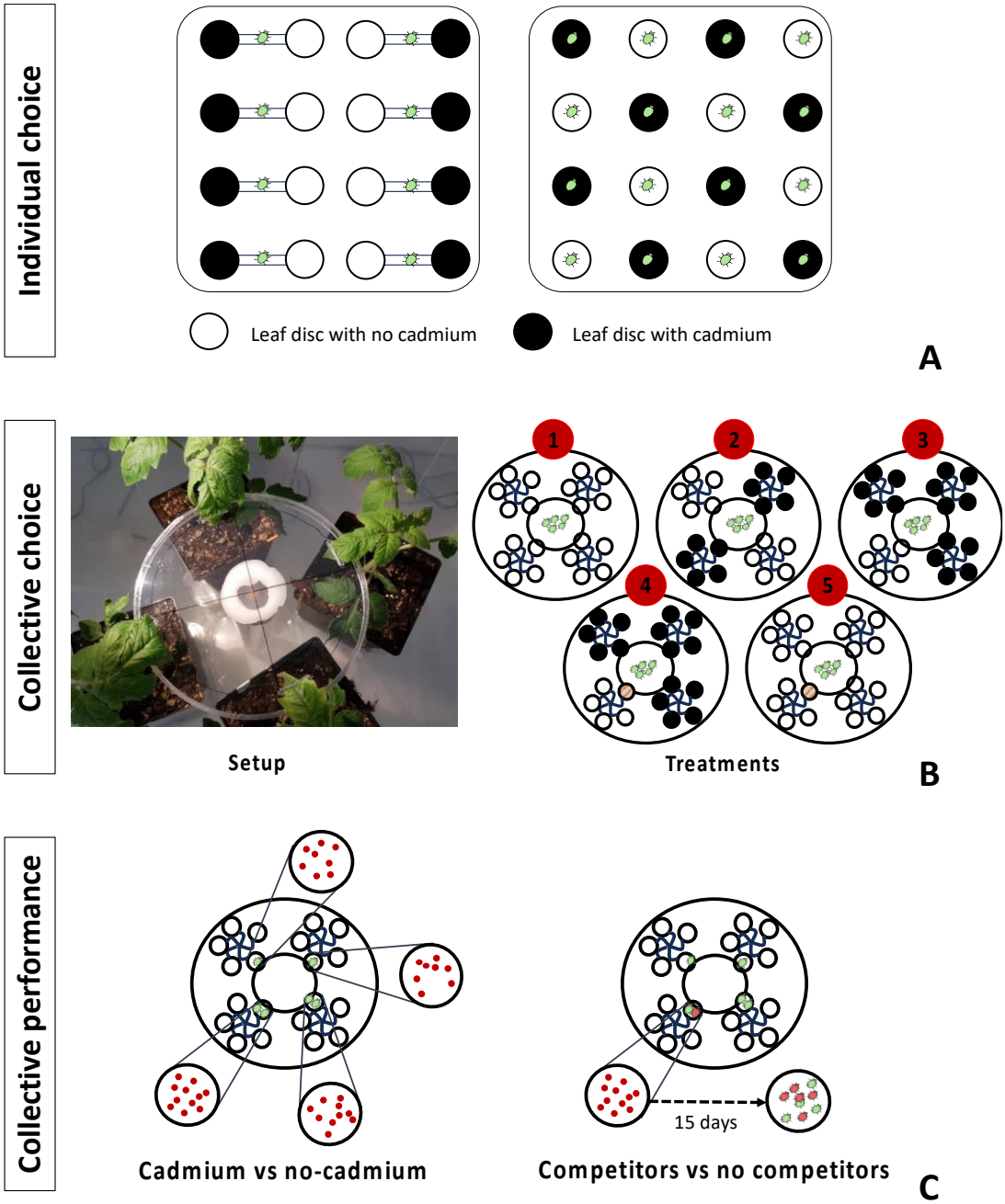

Figure 1. Experimental set-ups to measure individual (A) and collective host choice (B) and performance (C). The white and black shapes represent plants without and with cadmium, respectively. A) Experimental set-up for individual host choice (left panel) and individual performance (right panel). B)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

145 ***Experimental set-up for collective choice and performance***

146 To measure how the presence of competitors and the frequency of plants with cadmium affected the
147 collective choice of spider mites, four entire tomato plants were placed in a plastic box and were
148 connected by a round plastic arena (15 cm of diameter) in which their 4th leaf was inserted in a hole
149 of 2 cm long at equal distance from each other and from the centre of the arena (Fig. 1B). We applied
150 a ring of Vaseline around the petiole of the 4th leaf close to the node to prevent mite dispersal to other
151 leaves of the plant. We then released 200 females of *T. urticae* in the centre of the plastic arena, from
152 where they could move to any plant, and counted the number of mites on each plant 24 hours later.
153 This setup was used to test whether spider mite distribution was random or affected by the choice of
154 conspecifics (i.e., if there was aggregation), by giving mites the choice between four uninfested
155 cadmium-free plants (treatment 1; Figure 1B). Additionally, we used this set up to address how the
156 distribution of spider mites was affected by the frequency of contaminated plants and the presence
157 of a competitor in contaminated and uncontaminated environments. To address the first question,
158 we placed mites in set-ups with either two (treatment 2) or three (treatment 3) plants with cadmium
159 (Figure 1B), with the remaining plants being cadmium-free. To answer the second question, we
160 created two other treatments, in which the leaf of one plant without cadmium was infested with 100
161 adult females of the competitor species *T. evansi* (50 females on the first leaflet and 25 on the two
162 lateral leaflets to avoid overexploitation by the competitor). This plant was surrounded by three plants
163 either with (treatment 4) or without cadmium (treatment 5; Fig. 1B). The 200 *T. urticae* females were
164 placed in the centre of the plastic arena 24 hours after the installation of *T. evansi*. The number of *T.*
165 *urticae* females on each plant was counted 24 hours later.

166 To measure the performance of *T. urticae* on plants with or without cadmium, the number of
167 eggs on each leaf was counted on all plants in treatments 1, 2 and 3 after having counted (and
168 removed) the females to measure choice. The number of eggs was then divided by the number of
169 females on the same plant, to obtain the per capita oviposition rate. To measure the performance of
170 *T. urticae* in presence of competitors (treatments 4 and 5), we quantified the number of adult female

171 offspring after fifteen days, because the eggs of the two species are not distinguishable. To this aim,
172 we removed the females from the infested leaf of the plant with competitors (from treatments 4 and
173 5) or from one random plant without cadmium in the respective controls (treatments 1 and 3). Each
174 leaf was detached and placed in a pot with water in an isolated box. Fifteen days later, the number of
175 adult females was counted. Twenty replicates per treatment were done (except for treatment 5 for
176 which 19 replicates were done).
177

178 ***Statistical analysis***

179 Individual choice and performance

180 To test if individual females choose between cadmium or cadmium-free leaf discs, a general linear
181 mixed model with a Binomial error distribution was used. The dependent variable was the presence
182 or absence (coded as 1 or 0, respectively) of the female in each environment (using the cbind function
183 in R). To test the effect of cadmium on the female performance, the number of eggs laid on each patch
184 was analysed using a general linear model with a Poisson error distribution. The dependent variable
185 was the number of eggs per patch, the test environment (cadmium-free vs. cadmium leaf discs) was
186 coded as a fixed factor. For these models leaf age and replicate were used as random factors.

187 Collective choice and performance

188 We first tested whether spider mites distribute themselves evenly across cadmium-free plants. To this
189 aim, we performed a G-test goodness of fit per replicate, assuming an even distribution of mites across
190 plants (*i.e.*, 25% per plant). To control for multiple tests, a Bonferroni correction was applied.
191 Moreover, we performed a general linear model comparing the control treatment to a mock
192 treatment where each plant 'received' 50 individuals of the 200 released.

193 All statistical analyses to test the collective spider mite behaviour were done using a general
194 linear model with number of individuals on a plant as the dependent variable. All models included
195 block and interaction between identity of the plant and replicate as random factors. Models where

196 initially fit with a Poisson distribution, but due to overdispersion, a negative binomial error distribution
197 was used instead.

198 To test whether spider mites collectively avoided plants with cadmium, we compared the
199 distribution of females in plants with and without cadmium (treatment 2, 3 and 4) using a model with
200 cadmium and treatment as fixed factors and their interaction. Post hoc contrasts were performed to
201 test whether spider mites avoided plants with cadmium in each treatment, and to test whether
202 treatments differed concerning this avoidance. To test if cadmium concentration affected spider mite
203 choice we compared treatments 2 and 3, and to test if presence of competitors affected choice of
204 plants with cadmium we compared treatments 3 and 4. Finally, we tested whether the presence of
205 cadmium in the environment affected the avoidance of competitors by comparing treatments 4 and
206 5 with a model with competitors and treatment as fixed factors and their interaction. Post hoc
207 contrasts were performed to test whether spider mites avoided plants with competitors in each
208 treatment, and whether treatments differed concerning this avoidance.

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

213 We first assessed spider mite performance in the control treatment (i.e., treatment 1) to test
214 whether mite oviposition rate was even across plants, using a generalized mixed model with plant as
215 fixed factor. The effect of cadmium on spider mite performance was assessed by comparing the per
216 capita oviposition rate across all plants among treatments containing plants with and without
217 cadmium (i.e., treatments 2, 3 and 4). For that we applied a model with cadmium and treatment and
218 their interaction as fixed factors. Next, we performed pairwise comparisons to test whether spider
219 mite performance differed on plants with or without cadmium within each treatment. We also

220 performed pairwise comparisons to test whether the performance on plants with cadmium differed
221 across treatments, only using data from plants with cadmium in each treatment.
222 To estimate if the effect of cadmium was similar for the individual and collective tests, we
223 calculated the standardized effect size for each treatment and experiment using the effectsize package
224 (Ben-Shachar et al., 2020).
225 To test the effect of the competitor on the number of per capita offspring we used a model
226 with presence (or absence) of competitors as fixed factor. We used posthoc tests to compare the
227 treatments with competitors and their respective control (i.e. treatments 1 vs 5 and 3 vs 4).
228 All statistical analyses were performed using the software R v3.6 (R Core Team, 2022), using
229 packages glmmTMB and lme4 to perform the general linear models (Bates et al., 2015; Brooks et al.,
230 2017), car for the ANOVA tests (Fox & Weisberg, 2019), emmeans for post-hoc contrasts (Lenth, 2023)
231 and ggplot2 for visualization (Wickham, 2009).
232

233 **Results**

234 Individual choice and performance

235 Individual spider mite females did not occupy differently leaf discs with and without cadmium
236 (estimated overall probability = 0.0554, z ratio= 1.064, P = 0.2875, Fig. 2A). They laid significantly fewer
237 eggs on leaf discs with cadmium than on leaf discs without cadmium (ratio between water and
238 cadmium= 2.1, z ratio= 10.432, p<0.001, Fig. 2B, Table S1).
239

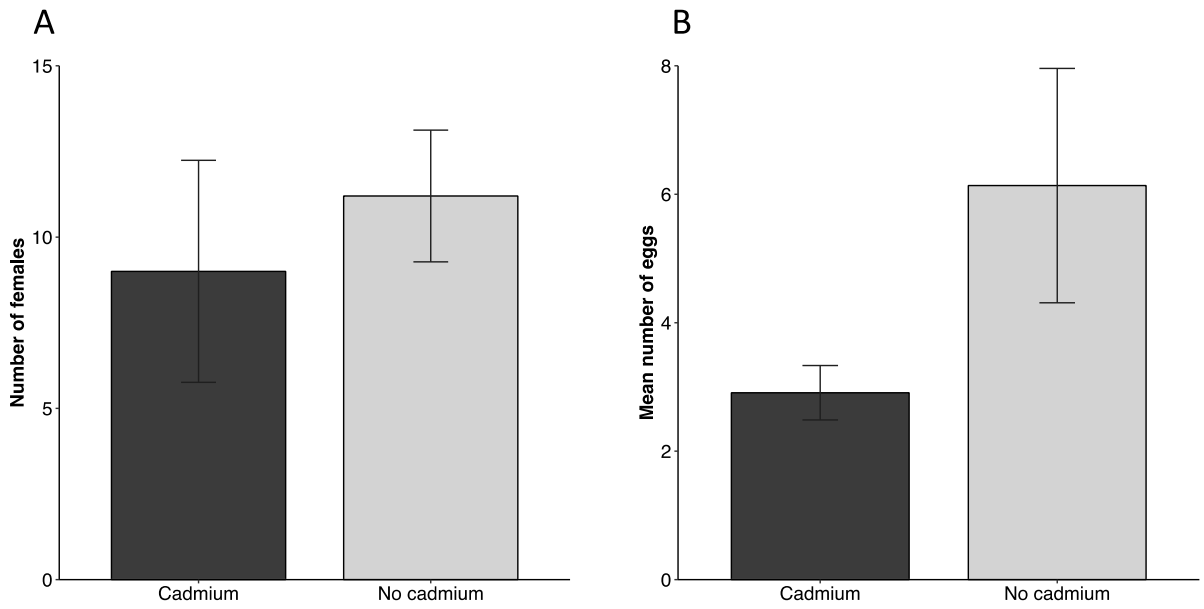

Figure 2. Individual host choice and performance

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

<u>Collective choice and performance</u>

Spider mites distributed themselves unevenly across plants in our set-up. In 18 out of the 19 replicates, they showed a distribution significantly different from the expected 25%, even after Bonferroni correction (Table S2). Indeed, the difference between the treatment with only cadmium-free plants and no competitors and a mock variable with an even distribution across plants was significant (effect of treatment: X^2_2=7410,5; P < 0.0001, Fig 3, treatment 1).

Cadmium significantly affected the number of females on each plant, but not treatment or their interaction (ANOVA results cadmium: X^2_1 =8.70, p-value =0.0031; treatment: X^2_1 = 1.678, p-value= 0.432; cadmium*treatment: X^2_1 = 1.674, p-value = 0.432; Fig. 3). Spider mites avoided plants with cadmium in all treatments (treatment 2 plants: z ratio=-55.987, p-value <0.0001, treatment 3: z ratio=-49.560, p-value <0.0001; treatment 4: z ratio=- 49.343, p-value <0.0001). This avoidance was

not different between treatments with two or with three plants (contrast: z ratio=-0.390, p value = 0.9196).

The presence of a competitor, and its interaction with treatment significantly affected the number of females on each plant (competitor: X^2_1 = 4.556, p-value= 0.033; treatment: X^2_1 = 1.666, p-value=0.197; cadmium*treatment: X^2_1 = 5.556, p-value =0.0184; Fig. 3). The number of mites on plants with or without competitors when all plants were cadmium-free did not differ significantly (contrast: z ratio=1.203, p-value = 0.229). However, in the treatment where plants without competitors had cadmium, females chose more often the plant with the competitor (i.e. they avoided cadmium), resulting in a significant difference among these treatments (contrast: z ratio= -2.134, p-value = 0.033). This choice was not different from that documented when the uncontaminated plant did not have competitors (contrast between treatment 3 and 4: z ratio=-0.457, p value= 0.891).

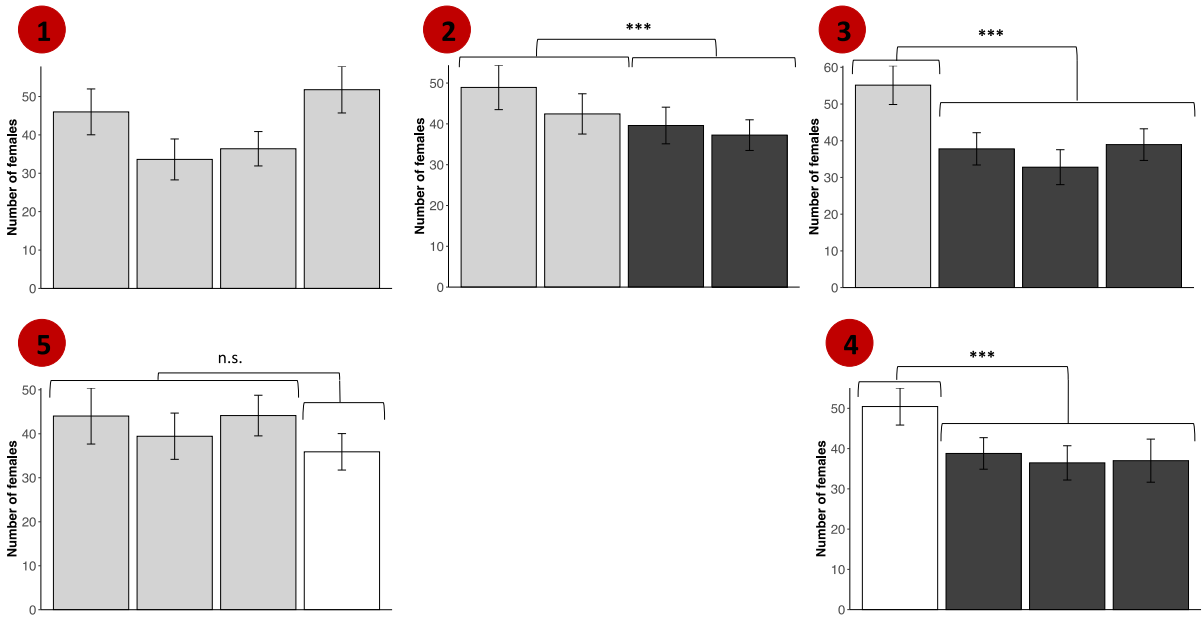

Figure 3. Collective host choice. Number of females after 24h on each plant for all treatments. Numbers indicate the treatments: 1) control, 2) two plants with cadmium, 3) three plants with cadmium, 4) one plant infested with competitors, 5) one plant infested with competitors and three plants with cadmium. Dark grey bars represent plants with cadmium, light grey colour plants without

cadmium or competitor, white bars represent presence of competitors on the plants. Error bars represent replicate standard error between replicates.

In the control treatment, spider mite oviposition rate was not significantly different among plants (X^2_3 = 3.473, p-value = 0.324; Figure 4). The presence of cadmium in plants, in treatments 2, 3 and 4 significantly reduced the oviposition rate of females (X^2_2 = 89.745, p-value <0.0001; Figure 4). However, the number of plants with cadmium did not affect spider-mite performance (X^2_2 = 0.413, p-value = 0.813; Figure 4). In fact, the standardized effect size associated with the presence of cadmium was remarkably similar for treatments 2 and 3 (Table S1). The effect size of cadmium was larger in individual than in collective performance experiments but showed a clear overlap of the confidence intervals (Table S1).

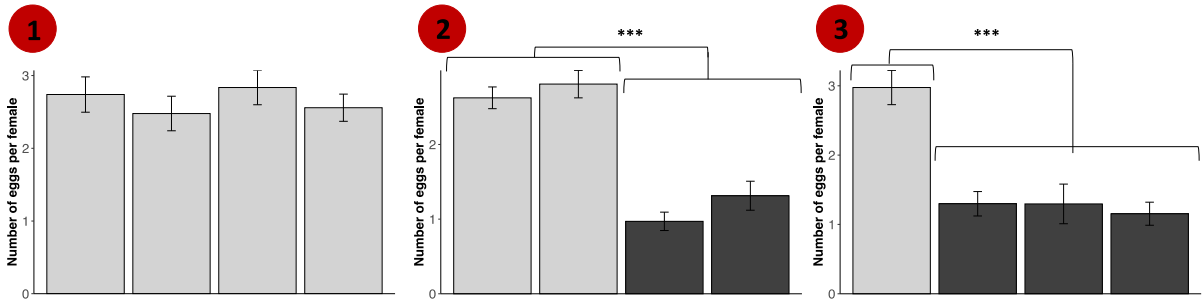

Figure 4. Collective performance. A) Number of eggs per female after 24h on each plant for treatments 1-3. Dark grey bars represent plants with cadmium, light grey colour plants without cadmium or competitor, white bars represent presence of competitors on the plants. Error bars represent replicate standard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

In environments with cadmium-free plants only, the presence of the competitor significantly affected the number of offspring per female after one generation (X^2_1 = 15.877, p-value = 0.0004, Figure 5). Contrasts between plants with or without competitors revealed that the number of female offspring on uninfested plants (*i.e.*, in the absence of competitors or cadmium) was higher than on

296 cadmium-free plants with competitors (contrasts between treatment 1 and 5: t ratio= 3.771, p-
297 value=0.002). However, this difference was not observed when competitor-free plants contained
298 cadmium (contrasts between treatment 3 and 4: t ratio= -1.713, p-value=0.325).
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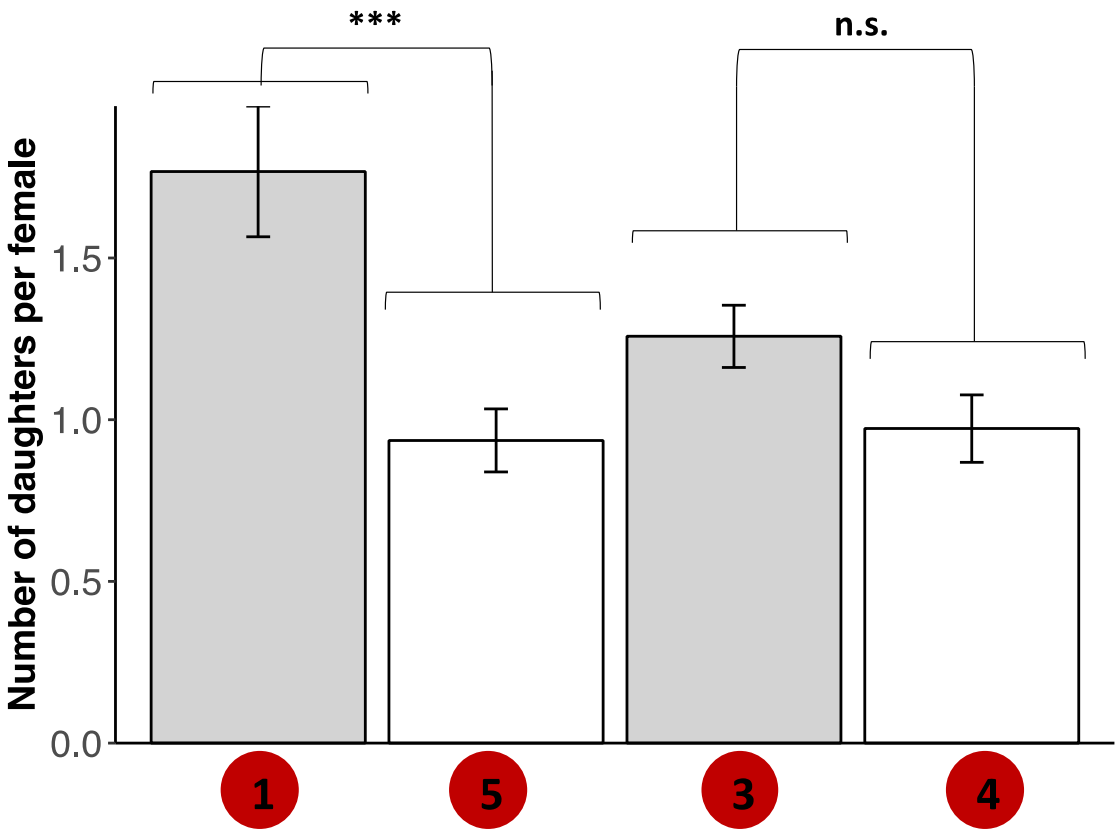

300
301 **Figure 5. Collective performance in presence of competitors.** Light grey bars represent plants without
302 cadmium or competitor, white bars represent presence of competitors on the plants. Error bars
303 represent replicate standard error between replicates. Both types of plants contained no cadmium.
304

305 **Discussion**

306 Our study shows that *T. urticae* females do not avoid tomato leaves with cadmium when they are
307 given the choice individually, although feeding on these leaves entails a reduction in their
308 performance. In contrast, when in group, facing the choice between cadmium and cadmium-free
309 plants, females moved more often to plants without cadmium. They also showed a non-even

310 distribution, even when all plants were uninfested and uncontaminated, suggesting aggregation
311 behaviour. Moreover, the frequency of cadmium-free plants had no effect on spider-mite avoidance.
312 Finally, when all plants were cadmium-free, spider mites did not discriminate between plants with or
313 without competitors, but they avoided plants with cadmium when the alternative was moving to a
314 uncontaminated plant with competitors.

315 We found here that spider mites feeding tomato plants that have hyperaccumulated cadmium
316 have a lower oviposition rate than mite feeding on non-contaminated plants. This recapitulates
317 previous findings (Godinho et al., 2023b; Godinho et al., 2018, 2022), and implies that they are robust
318 to the set-up used. Curiously, however, *T. urticae* females did not choose between leaf discs with or
319 without cadmium, a result also previously obtained for *T. evansi* (Godinho et al., 2023a). This is
320 surprising, not only because of the abovementioned consequences of cadmium for performance, but
321 also because this choice set-up had been validated previously (Rodrigues et al., 2017; Zélé et al., 2019).
322 Possibly, cadmium does not affect plant cues that inform spider mites of its content. Alternatively,
323 such cues may be produced in whole plants but not in cut leaf discs.

324 In contrast to the individual choice assays, spider mites systematically avoided plants with
325 cadmium in a set-up that simultaneously tested 200 mites. Our results from the control treatment
326 unequivocally show that spider mites do aggregate on particular plants, a result also found in the field
327 (Rijal et al., 2016). Aggregation behaviour has been shown to be beneficial to *T. urticae*, by increasing
328 fecundity and decreasing the death rate of females living in groups (Le Goff et al., 2010). In the
329 treatments containing plants with and without cadmium, such aggregation occurred on plants without
330 cadmium. It is thus tempting to hypothesize that spider mites use the information of their conspecifics
331 to avoid plants with cadmium. Spider mites often communicate by means of odour cues, for example
332 when searching for mates (Regev & Cone, 1976; Rodrigues et al., 2017) or avoiding predators (Choh
333 & Takabayashi, 2010; Janssen et al., 1999). Possibly, aversive chemical information of spider mites
334 that have been on plants with cadmium is available in our set-up and used by spider mites to make
335 choices that are beneficial for their performance. Alternatively, cadmium disrupts the production of

336 plant volatiles induced by spider mites, which are also used by conspecifics to find infested plants (Lin
337 et al., 2022; Pallini et al., 1997). In this case, cadmium-free plants are expected to become more
338 attractive than plants with cadmium.

339 In environments without cadmium, *T. urticae* spider mites did not avoid plants with
340 competitors h, which is at odds with competitors negatively affecting the number of offspring
341 produced, as found here and in other studies (Fragata et al., 2022; Sarmento, et al., 2011). Possibly,
342 avoidance of *T. evansi* is only observed in later stages of plant infestation, when resources become
343 scarcer. Indeed, *T. evansi* is known to downregulate plant defences, which is beneficial to *T. urticae* in
344 the short term (Godinho et al., 2016; Sarmento, et al., 2011). Moreover, *T. urticae* is able to avoid *T.*
345 *evansi* within a single plant (Godinho, et al., 2020a), which may release the selection pressure to avoid
346 whole plants.

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

357 The avoidance of cadmium even in the presence of competitors implies stronger competition
358 on cadmium-free plants. This is in line with studies showing increased intra or interspecific
359 competition in resource patches with higher quality in heterogeneous environments (Shen et al.,
360 2020; Wang et al., 2012). This suggests that spider mites will be exposed to different selection
361 pressures in heterogeneous environments: on plants with cadmium, selection will favour mites that

362 are able to physiologically cope with the toxic effects of metal, whereas on plants with no cadmium
363 mites will face stronger intra- and inter-specific competition. If these two selection pressures persist
364 for long enough, they may lead to high population differentiation within herbivores. Corroborating
365 this hypothesis would necessitate studying populations occurring on sites with both types of plants
366 (Freeman et al., 2007).

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

375

376 **Conclusions**

377 We demonstrated that spider-mites do not individually avoid tomato plants contaminated with
378 cadmium, despite having reduced oviposition on those plants. However, when choosing collectively,
379 they avoid cadmium contaminated plants, even when clean plants are colonized by a superior
380 competitor. These results suggest that collective choice in herbivores contributes to the maintenance
381 of plant elemental defences, as it entails less herbivory on those plants. Moreover, they imply that
382 hyperaccumulation of metals by plants may lead to stronger herbivore competition on nearby non-
383 accumulating plants, thus having broad effects on metal-polluted ecosystems.

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Author contribution

SM conceived the ideas and designed the methodology, with help from MCM; DG and MCM collected the data; DG, IF and SM analysed the data and wrote the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

Conflict of interest

Authors declare no conflict of interest.

Data availability

Data is deposited in Figshare, DOI: 10.6084/m9.figshare.24511093.

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