Peer Community In Ecology

# We are better together: Spider mites running away from Cadmium contaminated plants make better decisions collectively than individually

**Ruben Heleno** *based on peer reviews by 2 anonymous reviewers*

Diogo Prino Godinho, Ines Fragata, Maud Charlery de la Masseliere, Sara Magalhaes (2024) Spider mites collectively avoid plants with cadmium irrespective of their frequency or the presence of competitors. bioRxiv, ver. 4, peer-reviewed and recommended by Peer Community in Ecology. https://doi.org/10.1101/2023.08.17.553707

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Hyperaccumulator plants can concentrate heavy metals present on the soil in their tissues, avoiding their toxic effects and potentially discouraging herbivores (Martens & Boyd, 1994). But not all herbivores are necessarily discouraged, and access to locally abundant resources with low interspecific competition from other herbivores, can affect feeding choices. Godinho et al. performed a series of controlled laboratorial trials to evaluate if herbivores (spider mites) avoid tomato plants with high concentrations of Cadmium under alternative scenarios, namely: the presence/absence of conspecifics, the presence/absence of a competitor species (a congeneric mite), and the relative abundance of contaminated plants.

They found that when looking for plants to lay their eggs, individual spider-mites (females) do not seem to discriminate between plants with or without cadmium, despite a significantly lower performance on the former. However, they consistently chose plants without Cadmium in set-ups where 200 mites are faced with this decision together. This preference was consistent and independent from the relative abundance of cadmium-free plants, but only when mites do this decision collectively. In addition, this preference was stronger than that for plants where interspecific competition was lower, with mites preferring to face high competition from congeneric herbivores than laying their eggs on Cadmium contaminated plants.

Taken together these experiments suggest that aggregation is a key mechanism by which spider mites can avoid metal contaminated plants. As good research often does, these experiments open several important questions that will need to be addressed in the future. In particular, it will be important to clarify what are

the sensorial and behavioural mechanisms that allow this decision/outcome when spider mites make this choice collectively but lead to a different outcome (no choice) when they face this decision alone. Additionally, it will be interesting to explore the potentially adaptive (or non-adaptive) consequences of this behaviour in terms of individual and inclusive fitness. One thing seems certain: both the abiotic and the biotic context can affect spider mite choices, and both need to be considered to advance our understanding about the trade-offs between plant defence mechanisms and associated herbivore decisions and fitness.

***References:***


Martens, S. N., & Boyd, R. S. (1994). The ecological significance of nickel hyperaccumulation: a plant chemical defense. Oecologia, 98(3–4), 379–384. https://doi.org/10.1007/BF00324227

Godinho, D. P., I. Fragata, M. C. de la Masseliere, S. Magalhaes 2024 Spider mites collectively avoid plants with cadmium irrespective of their frequency or the presence of competitors. bioRxiv, ver. 4, peer-reviewed and recommended by PCI Ecology 2023.08.17.553707. https://doi.org/10.1101/2023.08.17.553707


# Reviews

## Evaluation round #2


DOI or URL of the preprint: https://doi.org/10.1101/2023.08.17.553707
Version of the preprint: 3


### Authors' reply, 17 June 2024

Dear recommender,
Thank you for your assessment of our manuscript. We have followed all your suggestions.

### Decision by Ruben Heleno, posted 11 June 2024, validated 11 June 2024

**Minor revisions**

Dear colleagues,

Thank you for the clarifications provided to the reviewers' and my queries. I feel that the message is clearer, including the figures, and that all relevant suggestions received during the first round of review have been sufficiently addressed. There are only a few very minor clarifications and corrections that should be implemented before the contribution could be recommended. I outline these below:

Line 177, Please indicate that males of the two species are not easily distinguishable, otherwise the readers might not understand why only females where counted.

Line 351, Maybe better to clarify that this sentence raises several potential explanations/hypotheses rather than known facts (add "can/might/could/potentially…" as you see fit).

Line 372, Remove additional parenthesis.

Please uniformize the hyphenation throughout the text in:

"set-up / set up"

"post-hoc / posthoc / post hoc"

"spider-mite / spider mite"

"inter-specific / interspecific"

Looking forward for a final, corrected version.
Best regards

## Evaluation round #1

DOI or URL of the preprint: https://doi.org/10.1101/2023.08.17.553707
Version of the preprint: 3

### Authors' reply, 06 June 2024

**Download author's reply**
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### Decision by Ruben Heleno, posted 28 January 2024, validated 29 January 2024

I have read the manuscript by Godinho et al. and also the reviews from two very competent experts in the field. Overall, we all agree that the manuscript contributes with relevant information to understand how mites select plants with lower levels of Cadmium. On the other hand, there are also a number of suggestions for clarifications and improvements that can considerably strengthen the conclusions of this work. I ask the authors to critically consider these suggestions when preparing a revised version of their manuscript.

My comments

This work reports on a set of well-planned experiments to clarify the individual and collective decision process of spider mites when faced with a selection of heavy-metal (Cadmium) contaminated plants and plants with high competition from interspecific herbivores. Both feeding preferences and reproductive performance on all treatments is analysed.

The question is relevant and has the potential to significantly advance the state-of-the-art. The manuscript is clear, and the experimental and statistical approaches are mostly well explained. One concern is that the actual presence of cadmium on the tested leaf disks should be further clarified, as from L323 it seems that the presence of cadmium on leaves disks of plants grown in the presence of cadmium has not been confirmed. Please clarify, this critical aspect of the experimental design.

The term "discriminate" on L21 might be confusing because although there was no significant choice on the presence of mites on cadmium and no-cadmium leaves, the former laid significantly less eggs on cadmium plants, showing that adults are capable of some form of discrimination, please clarify.

The significance of performing these tests with cadmium and not with other heavy metal, should be briefly presented in the introduction (more abundant? more widely distributed? critical effects? ...).

The results that individual mites don't differentiate between plants with and without cadmium, but they do differentiate between the two when in colonies is puzzling and I feel that it requires some further explanation. In particular, the fact that the choice is significant when in colonies, indicates that they have the individual physiological mechanisms to detect the presence of cadmium and that they probably also have an (individual) preference for cadmium free plants. Therefore, the reason that this effect is only statistically detected when in colonies but not on individuals (If I got it right), is probably because the collective behaviour intensifies the signal of individual choice mechanisms. Please clarify this or explain some alternative rationale.

Figure 3 The treatments Cadmium Vs. No-cadmium and competitors Vs, no-competitors are both identified with colour which make it more difficult to combine treatments (e.g. with cadmium and competitors). Consider using a more intuitive and informative way of indicating the treatments, for example with over imposed images or XX labels. Also, the font size is too small and hard to read. (similar issues on Fig. 4).

Figure 5 Maybe “daughters” is not the best term here, consider replacing with “emergences” or “offspring”. Please make the figure self-understandable by adding the meaning of the XX labels 1, 5, 3, 4 into the caption.

L306 (also on L377) Consider “… do not significantly avoid…”. Although statistical jargon should be avoided in the discussion, the true is that there is arguably some non-significant trend for a lower use of cadmium leaves, that could have been picked-up if there were more more replicates (?).

L315 “… feeding on tomato…”

L316 “…than mites…”

L380 The potential mechanisms enabling “collective choice” that are not present in “individual choice” should be further explored (if possible, by including known examples from other groups – if available).

## Reviewed by anonymous reviewer 2, 15 January 2024

I reviewed the manuscript titled “Spider mites collectively avoid plants with cadmium irrespective of their frequency or the presence of competitors” by Godinho et al.

The manuscript is in line with previous research line that the group has followed, to study the eco/physiological implications of cadmium accumulation in tomato plants, and the consequences on herbivorous mites.

This paper focus on the effect of cadmium-treated plants on the ambulatory dispersion of Tetranychus urticae, together with the presence or absence of the competitor species T. evansi.

Authors showed that meanwhile single mites do not made a selection between leaf discs coming from plants treated or non-treated with cadmium, the oviposition is affected negatively. On the other hand, when authors performed a choice assay using 200 mites, they found a clear preference for untreated plants compared with the treated plants, independently of the presence/absence of competitors mites. Authors concluded that cadmium accumulation by plants could enhance the herbivorous mite competition in heavy-metal free patches.

Introduction and discussion are well written and are adequate with the aim of the manuscript. Experimental setup and data analysis are appropriated for the research question. In my opinion the manuscript will improve with some text modification that clarify Material and Methods, and Results sections.

The use of the term “treatment” to describe the “layout” or different experiments performed in the manuscript is confusing. Authors should find another term to describe this factor.

I have some concerns on data presentation and statistical analysis.

- Related to data representation: Figures should be self-explanatory. Authors may represent with letters and/or asterisks the results of the statistical analysis in all plots. Authors should also provide enough information in the Figure legends related to the plot, including statistical analysis represented in each figure.
- Authors have to explain in more detail on statistical analyses related to collective choice experiment. Authors should explain how they handle the “missing” mites, i.e. mites that did not make a choice or were not find back on the plants.
- Figure 1C. Figure C follows a different layout related to cadmium treatment and make this plot very confusing. Authors should follow the same layout (color code) in all the plots. Did the authors move the cadmium-free plants to another setup to monitore the offspring? Authors should explain better the experimental procedure of this setup
- Line 202. According to material and methods, authors used one concentration of cadmium to treat the plants, so authors are not testing differences in “cadmium concentration”. Authors should find another term to describe this comparisson.

In this sense, authors did not show the output of this analysis in Results section. Furthermore, in my opinion the comparison between these two treatments will not give any extra value to the main observation of the manuscript i.e. spider mites avoid plants that accumulate cadmium. Uneven comparison can create an artifact. I mean, the comparison in layout 2 (50% control plants 50% cadmium treated plants) will be compared with layout 3 where the distribution of the treatments is uneven (25% control plans, 75% cadmium treated plants).

This could create a difference between the two treatments simply because in layout 3 the frequency of mock plants is lower, then it is expected an increase of mite density in clean plants in layout 3 compared to mock plants in layout 2.

- Figure 3. Panel 1. It seems there are strong preference for plants in positions 1 and 4 (bars aprox. 45 and 50 females respectively) meanwhile the other two positions (2 and 3) showed lower densities. Authors should include an explanation on this positional effect and how can affect the output of the assays.
- Figure 5. I understand from Material and Methods and Result section that this Figure represent the offspring of plants free of cadmium in treatment 1; 5; 3 and 4. Authors should describe if the average of Bar 1 corresponds to the average of 4 plants per repetition of the assay, or is the average of a single plant per repetition. In this case authors should explain which plant and in which position they selected to record the offspring specially since there are differences in mite densities depending on the position.

This Figure also shows that when there are plants treated with cadmium in the setup, the offspring is reduced (independently of the presence/absence of the competitor. Authors should include in the discussion an explanation for this observation. Are mites that end up in cadmium-treated plants moving to cadmium-free plants? This testing could impair the performance/offspring?

Other minor points (Authors should review the manuscript to refine the text and correct some typos)

Line 51. Add space between scarce and bracket

Line 193. Correct “general” to “generalized”

Line 226. Specify the posthoc test performed.

Line 340. Delete “h”

Line 248. Delete “even”

## Reviewed by anonymous reviewer 1, 24 January 2024

Plants can accumulate heavy metals from polluted soils, and herbivores would be affected by the bottom-up effect mediated by heavy metal accumulation. This research showed 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. This suggests that non-accumulating plants will suffer more from herbivore than accumulating plants in metal polluted environments. It is an interesting and meaningful research. I endorse the work and have a few suggestions for minor improvements, as listed below.

Abstract

The abstract is very long. I do not know if it is asked to show in five paragraphs. I suggest make it shorter and sharper. Then the authors should add some details of data from the results.

Introduction

This section points look good, but the paragraphs are too many. I suggest that some statements can be combined. L48-59, L67-L84.

Material and Methods

The authors should check and revise the format. For example, 48h should be 48 h.

L 102: the concentration of cadmium solution is 3mM or 2 mM? where did you treated with 3mM?

L 137: these experimental set-ups look good, but it can be mortified by adding border for each section to make clear.

Results

Fig.2 Statistical analyses results can be added in the figure to make clear.

Figures, the replications can be added in the legend, and the data points can be shown on the column. The treatments are not clear in the figure legends, I suggest that add details in the legends.

Discussion

L 340: “h”?

Have you checked the accumulation of Cd in tomato leaves? Does the treated ones did accumulate Cd and is that the reason for the results? Dose competition among the 200 individuals happen? Did they fight with each other?