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Prof. Rowan F. Sage

Chief Editor, Annals of Botany

3rd October 2024

Dear Prof. Sage,

**Major revision of Original Research article**

Please find attached a revised version of our manuscript ‘Functional biogeography of the thermal thresholds for post-dispersal embryo growth in *Conopodium majus*’, which we submit to your kind consideration for publication in Annals of Botany as an Original Research article.

We are very grateful for the chance to revise and improve our manuscript. We acknowledge the time invested in reviewing our manuscript by the handling editor Dr Hanley and two anonymous referees. Their comments have been invaluable and contributed to a better manuscript. Please find below our point-by-point response to the comments.

In my name, and in the name of my fellow co-authors (Cristina Blandino, Brith Natlandsmyr, Sylvi M. Sandvik, Hugh W. Pritchard), thank you very much for taking the time to consider our work.

Imagen que contiene vuelo, oscuro, noche, colorido

Descripción generada automáticamenteYours sincerely,

Eduardo Fernández-Pascual

Associate Professor of Botany

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**Editor Comments**

**The manuscript 24398 Blandino et al considers intraspecific variation in embryo growth thermal thresholds in the geophyte Conopodium majus throughout its European Latitudinal distribution (Iberian Peninsula to Scandinavia). Unsurprisingly, the authors report how thermal thresholds vary for populations across the latitudinal gradient with ceiling temperature, being the most important factor dictating embryo growth. This variable was additionally strongly related to the bioclimatic environment of each population, although optimal and base temperature were independent of local climate. The author conclude that these thermal thresholds are a functional ecophysiological trait driving seed germination phenology and, consequently, seed regeneration responses to the environment. Our Referees varied in their assessments of this submission, scoring the MS relatively well for the key AoB metrics of Originality and Priority, and recommending publication only after Major (Ref 2) and Minor (Ref 1) Revisions. Referee 2 was the most critical, but their comments concerning the overall framing of the manuscript and then how the important concepts that shape and advance the understanding of plant science are delivered in the Discussion, were echoed by Referee 1. Specifically, both Referees picked up on what they perceived to be a limited Discussion that did little to highlight any significance beyond implications for the species. It is essential that the authors highlight the wider message for plant science and keep in mind the AoB requirement that "we want to publish papers with novel and original content that move the subject forward, not papers that report incremental advances or findings that are already well known in other species."**

We are grateful for the general positive evaluation of our work by the editor and the two reviewers. We fully agree with your assessment that our discussion was too limited in scope. Therefore, we have revised our manuscript to implement all the suggested changes and, specially, we have rewritten the discussion to improve its generality and interest for a broad audience. In rewriting the discussion, we have paid extra attention to the specific points raised by the reviewers, which have been very helpful to identify discussion points to expand.

**The Discussion also needs to explain why this MS adds to Porceddu et al (2017 - cited in this manuscript) that applies a similar approach to embryo growth. It is insufficient to simply say you did the same thing in a different species or Family (see your Conclusions' section largely stating how this paper shows 'the same thing for an Apiacea' and then repeat core messages from the Discussion.**

We agree that the link with the Porceddu et al. work might not be relevant enough to be part of the conclusions. Therefore, in the revised discussion we have (1) moved it to the beginning of the discussion and (2) focused on the key message: Porceddu et al. found no variation (in the embryo growth thresholds) between populations, while we not only found said variation, but also found that it correlated with population climate.

**Please wrap your Discussion up in terms of the 'big picture, so-what' for plant science, not what could seem to be simply an incremental finding. Given the clear link to Climate Change, some brief consideration of that aspect is appropriate (see Parmesan and Hanley 2015 Annals of Botany 115: 849-864 - and other papers in the AoB Special issue on ACC and plants).**

We have fully rewritten the conclusions. Now, we do not focus on our study species, but on the wider implications for research on plant responses to climate change, and the key issues of phenology, distributions and evolution/plasticity.

**Consequently, I cannot recommend we publish this MS in its current form. Nonetheless, following strong revisions of the Discussion and consideration of my summary points and the detailed Referee comments, I am happy to reconsider a revised submission. Please use the Referee comments as a guide and when you submit your revision, provide a point-by-point response to the Referee comments in your Cover Letter. Without clear communication of wider novelty and substantial conceptual advance however ('....intraspecific variation in this key ecophysiological trait studied across the latitudinal species distribution - Iberian Peninsula to Scandinavia' has considerable merit - see Amstutz et al Pedobiologia 102: 150927), it is unlikely we can publish this submission.**

Please find below our point-by-point response to the referee comments. We have followed your instructions to produce a revised manuscript that makes a better job of presenting the novelty and the conceptual implications of our findings on functional intraspecific variation in the thermal thresholds for embryo growth. Thank you for the reference, we have incorporated it into the manuscript.

**Referee #1's Review for 24398:**

**The manuscript by Bandino et al conducted thermal time modelling of the Apiaceae Conopodium majus across a latitudinal gradient (from Spain to Norway). Ceiling temperature was identified as limiting factor for the embryo growth of these seeds with morphological dormancy, and it was found to correlate with latitude. Some comments to further improve this interesting work: MD as well as MPD was identified for Conopodium majus (Baskin and Baskin 2014; Blandino et al. 2019; Walker et al. 2021). Using the Baskin dormancy database Walker et al 2021 counted for the Scandicinae tribe to which this species belongs more literature reports for MPD than for MD. Blandino et al. (2019) reported MD, but all other known Conopodium majus and Conopodium spp. publications report MPD. Question: Do all your 9 seed lots have MD (and not MPD)? I think the MD/MPD point and that the Scandicinae tribe has mostly MPD should be discussed with also citing the phylogeny of Walker et al 2021. Would this (MD versus MPD) be associated with differences in the ceiling temperature, how is this adaptation related to latitude?**

The reviewer raises an interesting topic, and we thank him/her for pointing out the Walker reference, which we have integrated into our manuscript. Nevertheless, in this manuscript, we have purposedly focused on (1) the process of ‘*post-dispersal embryo growth’* and (2) describing said process in terms of thermal thresholds and requirements. It is not among our goals to label said process as MD or MPD in terms of the seed dormancy classification of Baskin and Baskin. A thermal description of the process offers more nuanced information - and a more accurate comparison between populations – than simplifying the process into two levels of a categorical variable. Specially, we note that we only use the term ‘*morphological dormancy*’ once in our text (P5 L5), and only for the purpose of acting as a reference for readers accustomed to the Baskin dormancy terminology.

**Calvino et al. (2016) discusses the role of the Southern Hemisphere in the evolutionary history of Apiaceae, a mostly north temperate plant family, which should be considered in the discussion.**

Thank you for this reference, which we have incorporated into our manuscript [P7 L10].

**To what extend is this finding (ceiling temperature - latitude) also important for breeding and how does it compare to Apiaceae crops (Walker et al. 2021; Zhang et al. 2019). I am not aware of thermal time modelling of Apiaceae crop seeds, but papers use something like 20ºC as germination temperature which for Conopodium majus is far beyond the ceiling temperature. Conopodium majus seems to like the cold, even in Spain it has optimal temperature below 5ºC. This is very surprising compared to Apiaceae crop seeds which may be worth a few sentences of discussion.**

Thank you for providing this interesting discussion point, we have revised our discussion to include a section about the comparison between our species and Apiaceae crops, and the general pattern of higher thresholds in crops vs. wild species (P20 L13-16).

**Also, from looking at table 2 it seems to me that all Norwegian, UK and 2 of the four Spanish accessions have very similar ceiling temperatures between 12 and 15ºC, while only two of the four Spanish accessions have a ceiling temperature >20ºC. Does this mean the "latitude correlation" is already evident in Spain alone? The two other Norwegian accessions are not too different from the 2 other Spanish accessions. Are CHO and TRE "exceptions"? This maybe something to include in the discussion.**

Thank you for this important observation. In fact, the accessions coded CHO and TRE belong to the subspecies *C. majus* subsp. *marizianum* (endemic to the western Iberian Peninsula), which differs from the typical subspecies C. *majus* subsp. *majus* (widespread in western and northern Europe). We have revised our discussion (P19 L6-13) to consider the biogeographical and functional implications of this fact.

**The references have some formatting issues. There are for example a few with titles in all capital letters.**

We have revised the format of the bibliography.

**Referee #2's Review for 24398:**

**The authors have applied the thermal time approach, well established for germination studies, to model low temperature embryo growth and estimate its cardinal points for a single species, across its latitudinal range. They have demonstrated significant intra-specific variation in these parameters in seeds collected from populations across a wide latitudinal gradient, with the upper temperature limit to pre-germination embryo growth being the likely most important limiting factor in controlling germination phenology in response to local climate. A strength of the research is that the authors have taken the (considerable) trouble to validate the lab-based modelling by field experimentation; though there are some unexplained inconsistencies. However, I am not clear why the fieldwork on seeds from three populations was only carried out at two locations - UK and Norway? Could not the responses of all three populations to the warmer, drier site in southern Spain have provided useful data for predicting possible effects of a warming climate on seed regeneration in Conopodium majus?**

We agree with the reviewer that this is a significant flaw of our study. We would have liked to perform a field experiment in our southernmost locations at the Sistema Central but, unfortunately, it was not feasible since the locations in which the seeds were collected are remote mountain sites, approximately 400 km away from the closest institution among our co-author team. Therefore, it would have been impossible for us to follow up the field experimentations. We have included a note on this in our discussion [P22 L12-14].

**If they have not already embarked upon it, the most interesting follow up study would involve reciprocal transplants of populations across those three sites; to establish how much of the variation measured and modelled is genetic and how much due to plasticity and/or maternal effects; focusing on the role for adaptation in any species' response to environmental change through seed regeneration.**

We are very grateful for this very interesting experimental idea. With the reviewer’s permission, we have added it to our discussion as a potential future experiment [P22 L17-22].

**p6, line 13. Strictly, these temperature parameters define just one aspect or dimension of Grubb's (1977) 'regeneration niche'.**

We have changed the sentence to ‘*Such narrow thermal control in a species with a relatively wide latitudinal distribution could make the species’ regeneration vulnerable to climate change […]’*.

**p9, line 24; use 'less than' instead of 'under'**

We have made the suggested change.

**p13, line 22 'traits'. Are they traits or 'parameters'?**

We have changed ‘*germination traits*’ to ‘*germination parameters*’ throughout the document.

**Prefer 'mean' instead of 'average' throughout.**

We have changed ‘*average*’ to ‘*mean*’ throughout the document.