

Dear Reviewer

On behalf of all authors let me thank for the considerations, comments and suggestions made to our submission.

We highly appreciate all the time and attention you have devoted to this submission but we cannot go on without letting you know how surprised and disappointed we were and still are, with the details of this second round.

Despite having agreed and complied with almost all comments and suggestions made by the two reviewers your overall evaluation of the submission worsened. Only the appropriateness of the research design, which has not changed, improved. The English worsened. The support provided to the conclusions by the results worsened and we are truly puzzled by it all.

A detailed point by point reply goes below in blue. After that a detailed point by point reply, also in blue, to all comments done in file *plants-422657_review#2.pdf* (all lines in it refer to the first version of the submission).

Sincerely
Luís

Open Review

- ☒ I would not like to sign my review report
- ☐ I would like to sign my review report

English language and style

- ☐ Extensive editing of English language and style required
- ☒ Moderate English changes required
- ☐ English language and style are fine/minor spell check required
- ☐ I don't feel qualified to judge about the English language and style

	Yes	Can be improved	Must be improved	Not applicable
Does the introduction provide sufficient background and include all relevant references?	☐	☒	☐	☐
Is the research design appropriate?	☐	☒	☐	☐
Are the methods adequately described?	☐	☐	☒	☐
Are the results clearly presented?	☐	☐	☒	☐
Are the conclusions supported by the results?	☐	☐	☒	☐

Comments and Suggestions for Authors

It is clear that there is plenty of work behind this document. The strengths are the wider ranges of heat shock treatments than other research and the variety of experiments. Most sections are good, yet substantial careful revision needs to be done to be suitable for publication.

I understand the data is old and probably the research has passed from hands to hands until the present and records were lost over the years.

Probably it was not intended but it is hard to read this sentence and not be struck by the not so disguised innuendo. Therefore a formal rebuttal is required.

Yes, the data is old.

No, the research has not passed from hands to hands. The research has always been in our hands from the very beginning until the present, 'our' meaning the three authors of the submission, and in our hands only.

No, no record was lost. Not a single one. If required we can provide hard copies of all records.  Let us know where to send them.

Seed collection and experiments started 30 years ago and conducted for at least 20 years. However, there is no evident objective for doing that reflected on the document, that may be as well be conducted on a single year collection considering the results.

We agree that there is no evident objective for doing that reflected on the document. Therefore in this aspect we reverted to our first formulation. Please let us know if we misinterpreted your statement.

It seems that a big part of the story is missing, hopefully, to be published too.

We agree that a part of the story is missing but it is not the time or place to tell it. However by now what we can tell is that if it was half as easy to publish that *Cistus ladanifer* seed coats are not impermeable to water and that heat shock does not break the seed coat presenting convincing experimental evidences of it as it is easy to publish the contrary without the slightest experimental support, we wouldn't be here now. 

Furthermore, the methodology does not mention how much of these 20-year data were organized, obtained and grouped to be analyzed statistically. Seed source information missing could be added if available to broaden perspective (ecological, biogeographical or at least as a record among others). Viability, for example, can vary within species, populations, and even the same plant in different years.

On viability, please see below.

There are inconsistencies with the number of replicates and I still cannot see controls.

In relation to inconsistencies please see below reply to comment to lines 97-100 and 464. As for controls, experiments were of four kinds: Only one treatment was done to elucidate whether or not seeds imbibed, and how, without heat shock and controls are meaningless here (Experiment 4, 5). Several treatments were done to evaluate the effect of various temperatures and two light regimes on final germination; there was no treatment that could be logically used as reference to compare the effects of others, thus no statistical control existed (Experiment 1)  Several temperature treatments were done to seeds, some qualifying as heat shock other not but a) no one can be singled out as the reference for no heat shock among those without heat shock, b) no one can be singled out as the reference for heat shock among those with heat shock, therefore no treatment could logically be used as reference to compare the effects of others (Experiment 2). Two or three treatments were done including a no heat shock, no extraction treatment and one heat shock or an extraction treatment where not only a control can logically be considered but also is self-evident (Experiments 3, 6).

The literature in the previous version was outdated, it has been improved but superficial bibliographic research shows plenty of references about *Cistus ladanifer* and heat treatments only, conducted in the last decades and

many more in other *Cistus* species. Some of those have been conducted fairly similar experiments and gotten similar results, (in this species, time for germination to start and end after heat shock treatment at different temperatures has already been recorded otherwise). However, the document fails to stress the value of having tested a wider range of temperatures or use the existing references to support the research.

Please see below reply to comment to line 260.

Methodology and the objectives tend to be rather subjective. It was partly improved from the last version, but it is not clear how most things were done and why. Part of those is missing perhaps because they are obvious to the authors but are not for the reader and not described to be replicable.

We moved all “why” to methods and we did so to accommodate the highly emphatic suggestion made by reviewer #1. We agree that this might create some difficulties when reading in the order that sections are presented. We disagree that the “how to do” is unclear and not allows its repetition.

There is no clear path from methods to results exposed or hint of replicability. There is no consistency on what is written in the methodology (replicates, treatments and species pooled or treated otherwise), and results. In the previous revision, it was noted as a general comment but looks not to be understood.

Apparently not. And we are not quite certain that we fully understood it now.

The responses for experiment 4 are well defended in the comments the to reviewer in the sense that it lets clear that seeds can lose material during imbibition. It shows familiarity and understanding of relevant literature.

However, it is not so in the document, the arguments are not as strongly defended there.

It is not but this issue is rather secondary in terms of the work done. In fact the only reason to mention it results from the publication of all data for this experiment. Otherwise there would be no reason to mention it, not even in the methods section.

The rationale of weighting ungerminated seed after all other had germinated and not conducting any type of viability test at any stage at least to discard it as factor for lack of germination is not clear. Viability was inferred and extended from other results but not tested at any stage. Imbibition occurs commonly in a portion of most seeds of species described as PY as in your results but is not exclusive of viable seeds. Unfortunately, without a test, results can only be inferred and can be biased towards the desired results.

As we see it the argument is that without a specific test of viability of non-germinated seeds biased conclusions can be reached. This can be true in general but not in this particular case. Otherwise all conclusions on the enhancing effect of heat shock on seed germination of *Cistus ladanifer* have to be rejected. Using only the three papers commented below we find that with an appropriate treatment it is possible to record 90% of germination after 27 days (Corral et al. 1990), 99.5% after two months (Valbuena et al. 1992), 93% after 42 days (Pérez-García 1997), 100% after 6 weeks (our work). Thus, necessarily between 90% (interestingly in the shortest period of incubation) and 100% of viable seeds in four independent studies. Unless we assume that somehow seeds used in all the very high germination assays were almost all viable and those used in assays with much lower germinations were almost all unviable, the only reasonable conclusion is that for all practical purposes seeds of *C. ladanifer* should be viewed as viable. We argue like this in the discussion of experiment 2 omitting results of others. If deemed necessary we can add them.

By the way, the water gap referred before opens by itself with heat, is not separated process, it just happens when heat is applied. The comment before was so that the process was not overlooked, and the authors could consider complementing the discussion. Comments are not meant to change the direction of an experiment that was long ago finished.

Water gaps might open with heat. Also with shaking. In any case there is no evident need of their opening by heat or by shaking (which we have not done) because water enters seeds without heat shock. Therefore we think that their introduction in the discussion would only serve to obscure it. 

Other suggestions:

Avoid imprecise expressions such as much more, clearly, undoubtedly, evidently etc. Scientific literature requires objectivity.

Done.

The number of references 99 seems excessive for the paper, several are hardly relevant.

In the resubmission it had 92. We agree that the number of references can be an important matter in printed papers, less so in online papers. We understand that this is a disputable matter and what is relevant for one person might be irrelevant for another and vice-versa. Anyway we cannot guess which references you feel are hardly relevant and without some type of concrete information we cannot proceed any further. After all we obviously considered all of them to be relevant otherwise we would not have made them.

The book of Baskin and Baskin 2014 has tables and databases with references to most seed publications in any area until recently. *Cistus* species, dormancy and treatments to break it are well referenced. Needless to say, it is a valuable resource by itself.

Needless to say. This matter will be addressed below.

Replies to comments in *plants-422657_review#2.pdf*

Lines 22 and 58-60: We don't have the 2014's edition of Baskin & Baskin's book (we assume that Baskin & Baskin 2014 refers to 2014's edition of *Seeds. Ecology, biogeography, and evolution of dormancy and germination*). The library of University of Évora neither. Albeit we have not done an in-depth search, 2014's edition seems to be also absent from libraries of a number of other Portuguese Universities or nearby Spanish Universities.  Even if we locate it somewhere, inter-library loans would take too long to be useful to this reply. However we have the 1998 edition and we will use as the best next thing. And we will obviously examine only references to *Cistus ladanifer*. In table 6.6 (Species in which dry heat overcomes physical dormancy of the seeds) *C. ladanifer* is listed and two references are presented to support such inclusion, which implies that those references should provide evidences that seeds of *C. ladanifer* have physical dormancy. Because these two references are also invoked (plus another one) in the comment to the manuscript in lines 58-60 and the question is the same we will examine the three references together.

We assume that Valbuena et al 1992 refers to *Int. J. Wildland Fire* 2, 15-20; Corral et al 1990 to *Seed Sci. Technol.* 18, 321-325; Pérez-García 1997 to *Plant Ecol.* 133, 57-62. We went through them, again, in search of "experimental support to the assumption that *C. ladanifer* seed coat is a barrier to water uptake by seeds". We will start with the more recent and follow the trail backwards to see where it leads us (bold type of our responsibility).

The only mention by Pérez-García to this issue is found in page 61, column b, lines 1-4, and we quote: "High temperatures **are supposed** to promote the cracking of the seed coat, particularly the palisade layer of macrosclereid with its strongly lignified cell walls (Corral et al. 1989)". Nothing more. Needless to say that "to be supposed" is not experimental support. And what has Corral et al. 1989 (*Phytomorphology* 9, 75-80) to do with it  Mostly the description of the seed coat. Perhaps also, and we quote: "The structure of the seed coat of the four

Cistus species investigated **might** constitute a serious obstacle to water absorption" (page 80, lines 6-7). It might. Or it might not. Where is the experimental support? Nowhere.

Valbuena et al. is a totally different case, because they only refer to this matter in the introduction, thus necessarily a second-hand approach, not their own. It happens in page 15, column b, lines 7-9 and we quote: "From these studies, it can be **deduced** that heat generally stimulates germination, by favoring the breaking of seed coats."

Please notice that they don't write "concluded" but "deduced". What studies are those? We can put them in three groups. Group A when no mention is made to any *Cistus* (Keeley 1977, 1987, and almost certainly also 1986 despite that we could only confer pages 95-99, 102, 103; Trabaud 1987a); Group B when mention is made to *Cistus* but not to *C. ladanifer* (Naveh 1973; Trabaud & Oustric 1989a,b); Group C when mention is made to *Cistus ladanifer* (Lopes 1998, which by the way was wrongly cited, and which we will transcribe in full: «As the propagation of the *Cistus ladanifer* L. takes place exclusively by seed it is / important to know in what conditions germination occurs. // Thus the impact of high temperatures (as in the occurrence of fire) and pre- / germinative treatments were studied. // The temperatures in which germination takes place vary between 7°C and 35°C / being the most favourable temperature to germination at 20°C and» (Book of abstracts, panel 65). Not a single mention to the "seed coat cracking" in *C. ladanifer* in the nine studies.

Finally Corral et al. 1990. In page 324, lines 4-6, and we quote: "According to Vuillemin and Bullard (1981) the seed coats in some species of *Cistus* would constitute an efficient barrier for water uptake, which might be overcome by dry heat treatments." No experimental evidence provided by the authors. What about Vuillemin and Bullard? The conclusions are questionable but no need to go deep into there. They did not study *Cistus ladanifer* (only *C. albidus* and *C. monspeliensis*).

Bottom line, there is no "experimental support to the assumption that *C. ladanifer* seed coat is a physical barrier to water uptake by seeds."

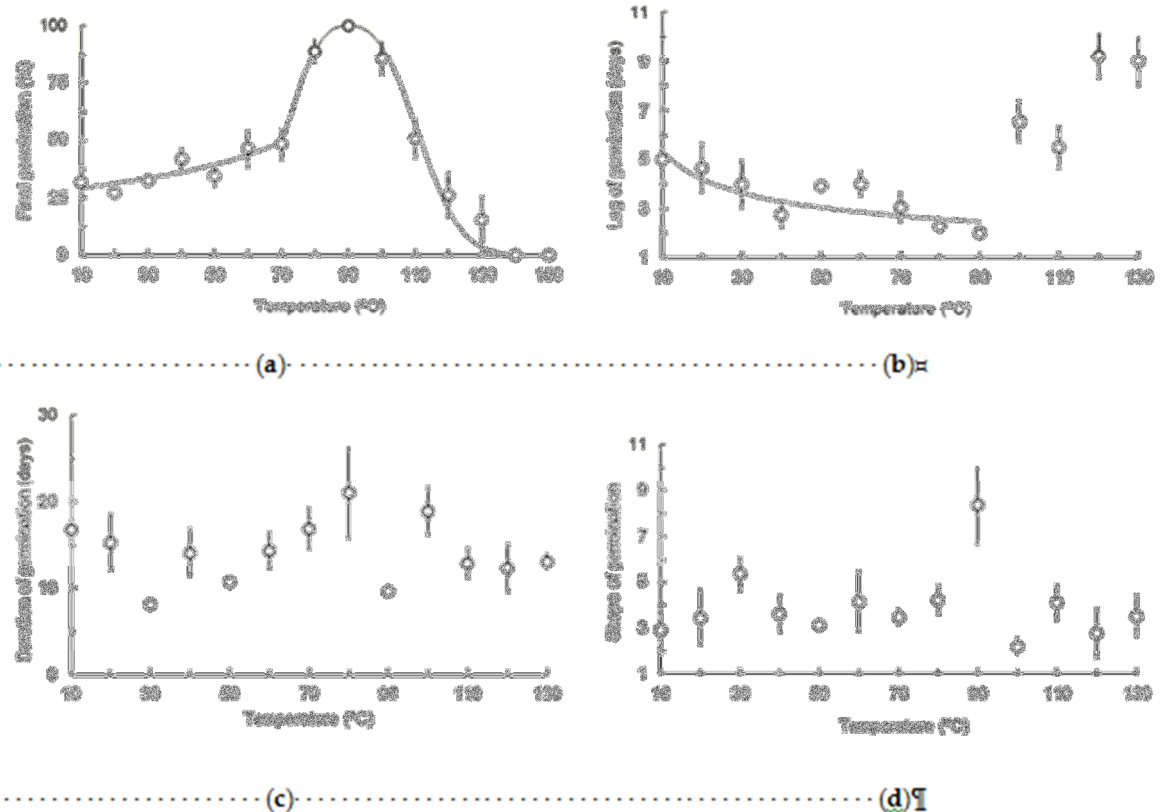
Line 71: It is now.

Line 71: Rephrased.

Line 72: We assume that you added and subtracted the means and the SE. Like is usually the case, they don't overlap, because a mean plus or minus the SE is a confidence interval in which the t-value is one. Sample size is $n=4$ in the two samples (table S1), meaning that their $df=3$, meaning that Student's $t=1$ and $df=3$ corresponds to a two-tailed probability of 0.391, thus to a confidence interval of 60.9%, and not to the confidence interval of 95% we adopted (comparison-wise type I error rates of 0.05 in the second line of section 4.6). However, for a 95% confidence interval of 95% and $df=3$, the t-value is 3.18. Thus the 95% confidence interval for the larger mean is 13.7-62.1% which encompasses the smaller mean.

Lines 88-91: Rephrased.

Figure 1 (Resize to fit): In the Word document the figure fits perfectly even with track changes activated. Somehow in the process of building the pdf it didn't fit anymore. Nevertheless if the paper is published (a huge if indeed) the figure will fit perfectly. Anyway we present it below.



Line 96: Confidence intervals are not error terms. Confidence intervals are SE (standard errors of the mean) multiplied by the appropriate Student's t-value and added and subtracted to the mean. SE are the appropriate statistical parameter to present with means either in text or in graphs.

Line 97-100: Please check again the last lines of section 4.2.2 and Table S2 where sample sizes are separately presented for all treatments. Also look at the sample sizes in germination data in Table S2. If all treatments had four replicates (=Petri dishes) the total would be 60 replicates. As it is, the total is 88 replicates. Of these, in 17 replicates no seed germinated and obviously no Weibull equation could be fitted (all 4 replicates in 140 and of 150 °C, 6 replicates in 130 °C, 3 replicates in 120 °C, thus 17), Weibull equations must be fitted by non-linear regression and anyone who has attempted to fit equations by non-linear methods is fully aware that sometimes no equation can be fitted especially when the acceptance of parameters depends on consistency checks of estimates and predictions against original data as explained in section 4.6. This failure happened in 18 replicates where germination had occurred. Thus Weibull equations could only be fitted and accepted in 53 replicates (17+18+53=88; please see also the sample sizes of Weibull parameters and estimates in Table S2). Anyway we rephrased this part as well as Table S2.

Line 113: Yes. In the regression analysis. No contradiction here because we are not dealing with regression but with the comparison of point estimates (means). Clarified in the beginning of section 2.2.

Line 118: It is a pooled mean for treatments which are not significantly different, not individual means which are those shown in the figure (the same for final germination, D_{100} , shape of germination).

Line 129: Eventually but a line (any line either straight or curve) requires at least one equation of any kind and in this case it would require arbitrary decisions on which would be the dependent and the independent variables. Obviously it could be attempted but we don't see the purpose of establishing a cause-effect relationship where none exist between the variables presented in the biplots, while the biplots as they are convey all the relevant information.

Line 165: We are dealing here with initial weights so there has been no size increase. Rephrased.

Line 165: Derived from table 8.10, p. 212. Rephrased.

Line 252: Done.

Line 254: Rephrased.

Line 256: The results are not ours. Rephrased.

Line 257: Done.

Line 257: Done.

Line 260: We disagree. This is important and complements existing information but is not “the main strength of this paper and difference from other research on this species already published”. The main strength and difference are showing that there is no evidence of physical dormancy in Baskin and Baskin (1998) sense 

Line 264: Done.

Line 420: Done. “Approximately” because the site suffered profound changes in the past 10 years or so. Vegetation was cut, new plantations were done.

Line 429: If seeds were harvested from two, maximum three neighboring plants (stated in the line immediately above) necessarily yes.

Line 431: No.

Line 432: It's easy to understand that it would be foolish to make experiments with seeds that could have been collected some 20 years before, unless the persistence of seed viability was being investigated which was not the case. A quite different question would be why did this work started so long ago and is still without having been published. We feel that a glimpse to the answer can be found somewhere above. 

Line 439: We disagree. Rietveld's criterion minimizes the chance of counting as functionally valid germinations abortive protrusion of seed coat without further growth. At least seedling growth has to overcome the largest dimension of the seed. It is meaningless whether ISTA or AOSA adopts this criterion  when seeds are subjected to lethal or potentially lethal treatments adopting Rietveld's criterion surely keeps us in the safer side.

Line 455: Rephrased but kept the reference.

Line 464: Detailed.

Line 768: Yes, we think so. The authors and the publishers decided that the title would be in three languages. It's not up to us to go against their decision, even if it was an erroneous (which is not the case) as happens in reference 3 where *salvifolius* is misspelled.