Dear Editor,

Thank you for the opportunity to resubmit our revised manuscript according to the reviewers comments. We have now re-assessed the “main points” raised by the reviewers and made substantial changes to the manuscript. Our understanding of the major revision points not previously addressed are the following:

1. Develop the rationale for choosing the temperatures in both experiments

2. Explain why timing is a factor in the experiments ( one is described as long term and the other as not long term) why?

3. Why have a range of temperatures ( first we look at cold and hot; second we look at five different temps for pollen)

4. Why are temps not the ones naturally encountered by plants.

We have made textual changes to the introduction and methods in particular in an attempt to clarify the issues raised by the viewer. In particular:

In regard to clarifying the rationale for designing the experiments as we did and incorporating the timing as mentioned -

The single broad goal of these two experiments is to test if thermotolerance to heat is higher in southern plants than in northern plants. Both experiments are designed to measure thermotolerance to extreme temperatures, but in different ways. The standard measure of thermotolerance is to measure breakdown at the cellular level for plant tissues exposed to an acute pulse of heat (or cold) extreme enough to partially but not completely destroy those tissues. That is the approach used in experiment one.

The second way we measure thermotolerance is to quantify the negative effects that (specifically) heat has on development of plants. The assumption is that thermotolerant individuals will have less of a negative response to growing in heat over a sustained period than will less thermotolerant plants. To get at that we grew plants in hot conditions and measured their floral and fruit development.

In the introduction we have made changes to the development of our rationale for looking at thermotolerance. In the methods we clarify further the goals of experiment one and two, outlining why they are different and how the two different measures of thermotolerance provide a fuller picture of how plants may have adapted in different parts of the plant range.

“In contrast to experiment 1, in experiment 2 we tested for differences in thermotolerance between northern and southern plants by measuring their developmental response to heat sustained over a longer period of time. The rationale for this experimental design is that the vulnerability of plants to relatively high temperatures may be more nuanced than complete tissue or cellular breakdown (as tested in Experiment 1). Instead, a thermotolerant plant may be one that can continue to develop normal tissues (e.g. flowers) over a sustained period when exposed to high temperatures.”

“Emma what would you think of changing the diagram to match the new experiment titles? I am thinking we could clarify why we did the two experiments by naming them differently”

In regard to the temperatures chosen for Experiment one and two:

Our goal was not to define the natural limits of what plants have encountered in the different environments. Rather we are quantifying the percentage of complete breakdown of physiological systems when they are exposed to a sub-lethal extreme temperature. If we exposed the plant tissue to boiling temperatures, it is certain all cell membranes would be destroyed, all pollen would fail to germinate and all chloroplasts would be destroyed along with the chlorophyll. However, that would tell us nothing about differences between northern and southern plants. Thus, we need extreme temperatures that will destroy these three physiological components but not completely. We chose intermediate temperatures (55C for example) to get intermediate estimates of breakdown that should then be comparable between tolerant and less tolerant plants. It is the equivalent of using intermediate temperatures to produce a sensitivity curve. We argue a tolerant plant is one with a shallower slope in a sensitivity curve. Using these temperature is standard practice for estimates of thermotolerance in plants regardless of the species or then environment from which they are derived.

We have updated the methods to include a more detailed explanation on the techniques used to assess thermotolerance in Experiment one.

We chose to examine both cold and hot temperatures in Experiment one to better understand the nature of any potential adaptation of plants to heat by testing the possibility that there is a tradeoff between cold tolerance and heat tolerance. If selection for tolerance of relatively extreme temperatures will lead to genetic divergence and consistent differences in tolerance between plants from different climates then plants in colder conditions should be more tolerant of cold. If there is a tradeoff between cold and heat tolerance then cold tolerance should be associated with reduced heat tolerance. Experiment 1 was designed to test both of these concepts. Climate change is expected to lead to increases in heat rather than cold but understanding how plants will respond may depend on both cold tolerance and heat tolerance.

There was some confusion about why we used a continuous distribution of temperatures from cold to hot for measuring pollen germination and tube growth (Experiment 1). The reason is that we wanted to quantify thermotolerance of pollen by describing the slope of a sensitivity curve, a powerful measure of how pollen responds to temperature. A curve allowed us to calculate derivative variables such as the maximum point of pollen germination and the maximum temperature required to completely disrupt pollen function. We felt the methodology developed by Gajanayake et al. (2011) was a more nuanced measure of thermotolerance.