**Title:** On Thermal Time and Cardinal Germination Temperatures: A novel analysis method using R

**Running Head:** Thermal Time & Cardinal Temperatures in R

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**Abstract (250 max)**

**Introduction**

Germination is the transition from seed to seedling. This transition occurs in response to a series of environmental cues, including temperature, moisture, light and chemical signals (Bewley et al 2013). For each of these cues, there is an optimal value at which the rate of germination is maximal. Below and above this optimum, the rate progressively decreases until the cue reaches base and ceiling thresholds beyond which germination stops. Thus, the rate of germination as a function of temperature can be described in non-dormant seeds as an accumulation of degrees-day above or below these thresholds (Garcia-Huidobro et al 1982). When a certain amount of degrees-day has been accumulated, the seed germinates. Every seed will require its own amount, but since experiments need to be done with a seed population, thermal time is expressed as the degrees-day needed to reach germination in a percentage of this population, usually the 50 %. The measuring of the germination cue in degrees-day, i.e. in thermal time, has the advantage of integrating time and temperature (Romo and Eddelman 1995). Therefore, the thermal control of germination can be described in a mechanistic way using a handful of seed traits: the three cardinal temperatures; sub-optimal (base Tb), optimal (To) and supra-optimal (ceiling Tc) temperatures and the thermal time. Thermal time traits have two powerful applications. First, parameters calculated in a restricted set of experimental treatments can give good estimations of germination in more complex thermal environments (Hardegree et al 1999). Second, thermal time parameters can be compared in a standard way across seed individuals, populations and species (Trudgill et al 2000) . As such, thermal time models provide the broadest base to conduct comparative studies and meta-analyses of germination (Dürr et al 2015).

Here, we present a novel method to calculate thermal times models and cardinal temperatures. This method is superior to methods currently being used in two ways; 1. The analysis is computed rapidly, in contrast to the hours or days it may take using alternative methods, and 2. This script identifies the breaking point in the data methodologically, rather than the user estimating breaking points on a case-by-case basis which is not ideally scientifically reproducible. We test the efficacy of using the method we have developed here, for calculating and identifying thermal time and cardinal temperatures across two experiments; one in which cardinal temperatures can be identified, and one in which experimental results did not lead to the successful identification of cardinal temperatures. We present here, the format in which the data should be set up (Supplementary Information 1), the script to analyse (Supplementary Information 2) the data facilitating the method we put forward, in an ideal and not ideal dataset, so that out method may be completely transparent and rigorously reproducible (Rocchini and Neteler 2012).

**Materials and Methods**

Data was collected according to time in days, and cumulative germination across 43 days (6 weeks) (Supplementary Information 1). All analysis has been performed in the R language and environment for statistical computing (R Core Development Team (n.d.)) and all plots created using the ggplot2 package in R (Wickham 2009) (Supplementary Information 2). First, the package *dplyr* (Wickham and Francois 2016) was used to group data by germination treatment and replicate, and then filter the data into a new file to only represent the results of the final scoring date. Then, a function was created to estimate the mean proportions of germination percentage, and the package *binom* (Dorai-Raj (n.d.)) was used to estimate the binomial confidence intervals of the total mean germination, and *dplyr* used to apply the function per treatment. The first plots represent total mean germination of each treatment, plotted against temperature treatment (Figure 1). At this point, the *Figure 1* was visually inspected for evidence that the temperature treatments resulted in representing the germination temperature range of each species. Where no sub or supra-optimal temperatures were achieved, the segmented model we present here, cannot be fitted, but just half the model to identify one half or the other.

The package *drc* (Ritz et al 2015) was used to fit a dose-response model, and test model selection for the best fit of different cumulative germination functions. Then the model with the best fit was used to plot the cumulative germination of each treatment against time, to check the functions fits visually (Figure 2). Next, a function was set to fit the calculated cumulative germination model to each treatment, to calculate the estimate and standard error, or each treatments effect on germination times. The times were then divided by one to calculate the germination rate of each treatment, for time to germination, for ten deciles 10-90%.

Where sub-optimal and supra-optimal temperatures were available in the data set, a segmented model could then be tested (Figure 3a). The package *segmented* (Vito (n.d.)) was used to set a function to fit a segmented regression and compute the intercept and the slope for each segmented relationship in the model. Where the data fits the model, there were two segments, one for the sub-optimal temperatures, and one for the supra-optimal temperatures (Figure 3a). Then, base temperature (Tb) was calculated by dividing the intercept by the slope of the suboptimal temperatures model, the ceiling temperature was calculated by dividing the intercept by the slope of the supra-optimal temperatures model, and optimal temperature was found where the two models intersect. Then, cardinal temperatures were calculated for each of the ten decile time to 10-90% germination. The fitted line for each segmented relationship were tested with the germination rate plotted against temperature for every decile.

Where the data does not achieve to identify one of either the sub-optimal or supra-optimal temperatures, a segmented model may not be tested (Figure 3b), but a standard linear regression can be used to identify one range of temperatures. We plot the germination rate against each temperature treatment using a smooth linear model for each decile in Figure 4.

**Results**

In *species A*, we achieved identifying the full range of cardinal temperatures (Figure 1, 2, Supplementary Information 3 Table S1), identifying a range of temperature rates (Table S2), where a segmented model could be fit to the data (Figure 3a), and the cardinal temperatures successfully identified (Table S3). In *species B,* the full range was not successfully identified (Table S1), but only the sub-optimal temperatures were achieved (Figure 1, 2) and the supra-optimal temperatures were not identified, as after the best germination rate was reached, the other temperatures did not successfully germinate at all and had a total cumulative germination of zero (0). In *species B* the segmented model could not be fit to the data (Figure 3 b), but could be fit to a smooth linear model to identify a potential portion of the sub-optimal range of germination (Figure 4 b).

**Discussion**

This method is appropriate for the use of the analysis of germination data to identify thermal time and cardinal temperatures. The method we present here offers an opportunity to calculate this analysis rapidly, and to select the breaking points in the data scientifically. When using this script, we recommend that users become familiar with the basics to using R, and seek out one of the many introductory tutorials that exist online. We suggest to ensure all packages are installed, and libraries uploaded, and follow the notes in the script using the example data sets in the supplementary information , using this paper as a complimentary resource to the script.

To engage in an experiment that will set out to identify cardinal temperatures, one must plan an experimental design which will purposefully identify inappropriate temperatures, which are below the optimal temperature (sub-optimal) and above the optimal germination temperature (supra-optimal), pushing each species to its polar limits. In order to fit a segmented curve, there must be a minimum of three suboptimal temperatures, and a minimum of three supra optimal temperatures. It is thus recommended that experimental design tests a minimum of 5-6 temperatures for each to effectively explore potential germination temperature ranges. The less that is known about a given species, the more temperatures that should be tested.

Constant temperatures are known to produce promising results for testing cardinal temperatures, particularly for agricultural varieties which have been bred for uniformity, and stable germination temperatures (Covell et al 1986, Ellis et al 1986). However many wild species, particularly alpine species, may require alternating temperatures, or alternating temperatures may better represent real ambient conditions found in nature (Carol C Baskin and Baskin 2014). When testing with alternating temperatures, it is recommended for plotting that the average temperature be taken of the two alternating temperatures for each treatment (Ellis and Barret 1994). Thus, alternating temperature regimes should be carefully planned to still result in temperature gradients when averaged, and may lead to odd results. Constant temperatures should never be compared to alternating temperatures within the same analysis, but if testing both, the two temperature regimes should be set up as two experiments, to compare the groups against each other. Ideally, in setting up an experimental design for wild species, a regime of both constant and alternating temperatures could be tested separately, and the results compared. Experiments are recommended to be run for four to six weeks, or when dormancy is present extended until #### (should we make a brief statement on how to account for, discredit, or allow for dormancy here?).

We have found and confirmed that this method is useful to calculate thermal time and cardinal temperatures in germination experiments which have tested a full range of temperatures across a given species germination gradient, both rapidly and rigorously.

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**Figure 1:** Final Germination proportions across all temperature treatments for species A) and species B)



**Figure 2:** Cumulative Germination Curves across all temperature treatments for species A) and species B)



**Figure 3:** Time to germination across each decile (%) of total germination, across each treatment using a segmented model for species A) and species B).



**Figure 4:** Time to germination across each decile (%) of total germination across each temperature treatment, using a smooth linear model for species A) and species B)