

# Effect of temperature on the germination and growth rate of Thale Cress (*Arabidopsis thaliana*)

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## Abstract:

Knowledge about the impact of temperature change on plant germination and growth is of growing importance in our current era of global warming and climate change. This knowledge is important because climate change has the potential to both disrupt ecosystem stability and to diminish global food production. This experiment sought to add to this knowledge about the impact of temperature on plants by investigating the germination and growth rates of the model organism *Arabidopsis thaliana*, under conditions of 12°C, 20°C, and 29°C. The results showed a statistically significant decrease in the germination and growth rates of the 12°C and 29°C plants when compared to those of the 20°C plants. The impact of temperature on gene expression, hormone levels, and chemical kinetics are discussed as potential causes for the decreased germination and growth rates. The experimental data lead us to provide support for the alternative hypothesis that temperature variation from the optimal have a negative influence on the germination and growth rates of *A. thaliana*. A variation of growth chamber light intensity is discussed as a potentially substantial source of error for the experiment.

## Introduction:

*Arabidopsis thaliana*, commonly known as ‘thale cress’ or ‘mouse-eared cress’, is a fast growing annual plant that has become the organism of choice for research in botany (Meinke *et al.* 1998). They are distributed widely around the world and grow to be approximately 10-40cm tall, forming one slender stem with a rosette of basal leaves (Meinke *et al.* 1998).

Recently, there has been an increase in spring temperature in the temperate zones, which is causing changes in many organisms’ developmental behaviors, such as growth and flowering (Blázquez *et al.* 2003). These changes have been correlated with chemical reaction rate, activity levels of proteins, hormone concentration, and body shape (Henderson and Dean 2004, Kaplan *et al.* 2004). Research into the impact of temperature changes on plants is especially important due to the twin phenomena of global population growth and climate change. The world population is

presently forecasted to reach 9 billion by the year 2043 (UN 2010). With this growth comes an accompanying need to maximize plant productivity in order to meet food demand. Concurrent with population growth, the planet is also experiencing climate change which threatens plant productivity, ecosystem function and food supply. Therefore, an investigation of temperature effect on a model organism such as *A. thaliana* is important for the analysis of temperature-induced behaviors in other plants.

Our null hypothesis ( $H_0$ ) states that a temperature variation, either an increase or a decrease from an optimal temperature, within the viable temperature range for *A. thaliana* (ecotype Landsberg *erecta*), will either increase or have no effect on *A. thaliana*'s germination rate and growth rate. The alternate hypothesis ( $H_a$ ) is that a temperature variation, either an increase or a decrease from an optimal temperature within *A. thaliana*'s viable temperature range (Blázquez *et al.* 2003) will decrease *A. thaliana*'s germination and growth rates.

Our alternate hypothesis is supported by Blázquez *et al.* (2003) who have found that the temperature that *A. thaliana* was exposed to affected its growth and flowering rates. Blázquez *et al.* (2003) found that at an optimal temperature of 23°C, the flowering of *A. thaliana* was faster than at a lower temperature of 16°C. Our alternate hypothesis is also supported by Li *et al.* (1998) who sampled 40 ecotypes of *A. thaliana* from a wide range of latitudes, and found that plants from higher latitudes and cooler temperatures tended to be of smaller size than plants from lower latitudes and warmer temperatures.

## **Methods:**

In this experiment we examined the impact of temperature on *A. thaliana*'s germination and growth rates by using three temperature intervals within a range of temperatures which *A.*

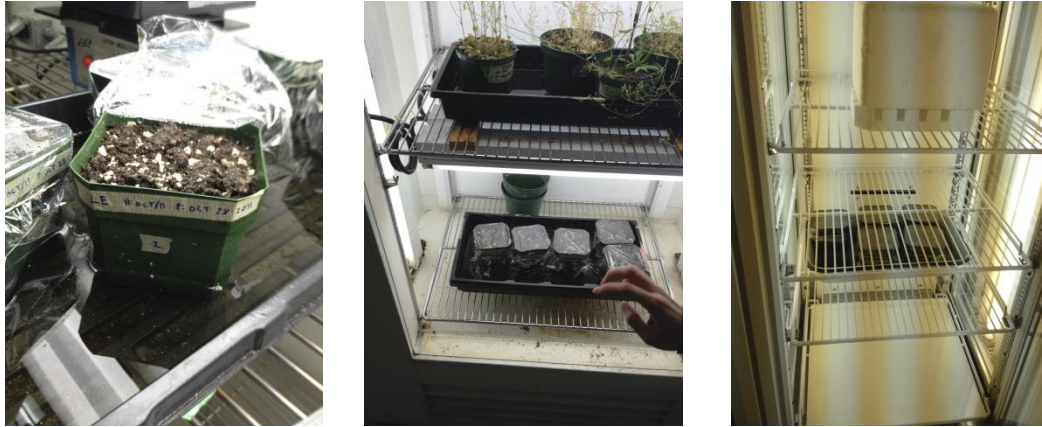
*thaliana* would typically be exposed to: 12°C, 20°C, and 29°C. The 20°C treatment was used as our treatment control as it was as close as it was possible to get to the optimal growth temperature of 23°C identified by Blázquez *et al.* (2003) within our equipment constraints. In this experiment, we will refer to the “optimal temperature” as 20°C. The 29°C treatment created a heat stress environment for *A. thaliana* (Kurek *et al.* 2007). The 12°C treatment created a cold stress environment for *A. thaliana* (Chinnusamy *et al.* 2007). Each treatment consisted of five small pots within which approximately five *A. thaliana* (*Landsberg erecta*) seeds were planted, for a total of approximately 25 seeds at each treatment temperature.

First we filled the pots with soil, tamped the soil down, and placed the pots in a drain tray, which was filled with 2.5 cm of water. The following day, we checked the soil by touch to ensure saturation. Then we put a planting board with multiple holes punctured into it over each pot to aid in the even distribution of the 5 seeds (Figure 1). A fine paintbrush was used to aid in seed placement.



After planting, we covered each pot with plastic wrap, and labeled the pots with seed identification information and date of planting. We placed one tray of 5 replicates in each of the growth chambers: 12°C, 20°C, and 29°C (see Figure 2). Once the seedlings had grown tall enough to contact the plastic wrap we removed the covering to allow the plants to access more carbon dioxide.

**Figure 1.** Planting of *A. thaliana* seeds with the aid of a punctured planting board to avoid crowding of seeds



**Figure 2.** Planted pots of *A. thaliana* are shown in their growth chambers: 12°C, 20°C, and 29°C respectively. Each chamber received 16 hours of light of different light intensities daily. The 12°C growth chamber received 4900 lux, 20°C chamber received 4300 lux, and the 29°C growth chamber received 3500 lux of light.

To ensure equivalent water supply, we kept the water level in each tray constant at 2.5 cm. All other factors were kept constant with the exception of light intensity, which due to equipment constraints varied between growth chambers. This variation is discussed as a potential source of error.

We collected germination and growth data for each treatment by recording the number of days it took for the seeds to germinate and measuring the height of the seedlings. Height data, to the nearest 0.05 mm was taken with calipers. Data were collected on days 3, 5, 7, 10, 12, 17, 19, and 21 after planting. Height data was not collected until the plants were approximately 2 mm tall to get a measurement without risking damage to the plants. Mean time to germination, with 95% confidence intervals for each treatment was graphed as shown in Figure 3. Mean daily heights, standard deviations, and confidence intervals for each temperature were calculated. A grouped scatter plot of the mean daily heights by temperature treatment was plotted with 95% confidence intervals as shown in Figure 4.

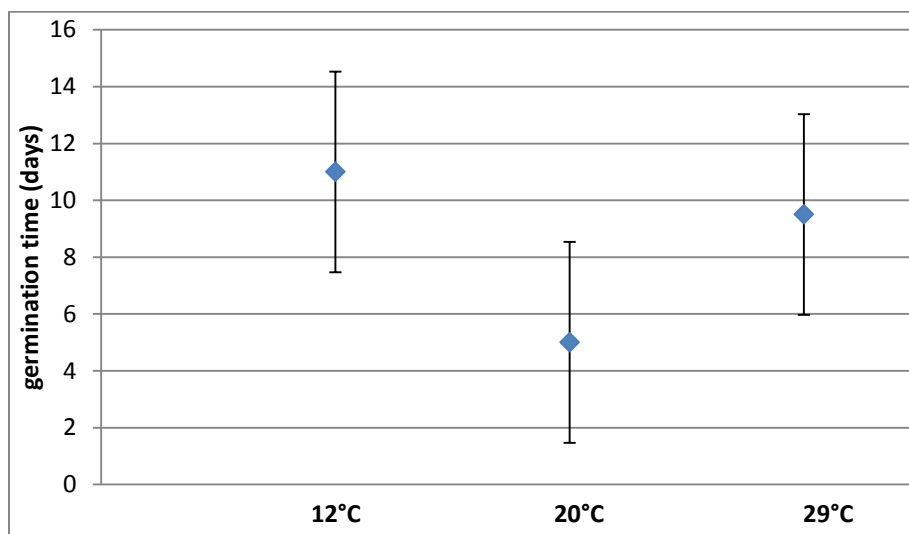
## **Results:**

Three major trends can be observed from the experimental data. As can be seen by examining Figures 3 and 4 (below), one major trend observed was that both sub-optimal

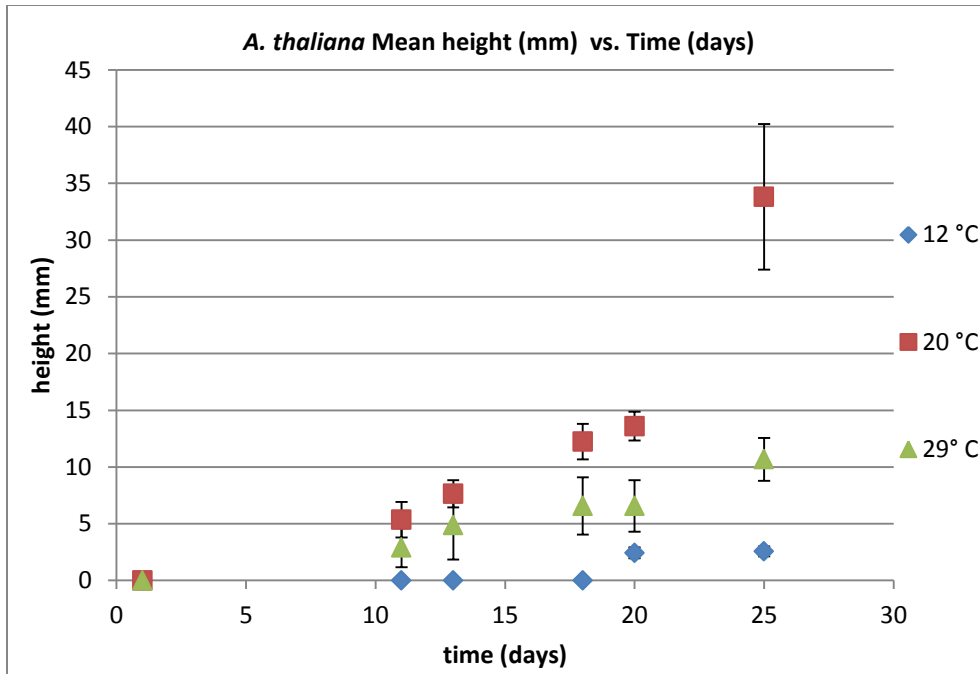
temperature variations: 12°C and 29°C exhibited lower germination and growth rates when compared to the 20°C treatments. Figure 3 shows that the 29°C seeds germinated 4.5 days later and the 12°C seeds germinated 6.85 days later. These differences are not significantly different. Figure 4 shows that the control plants at 20°C grew to significantly greater heights than the plants grown at 12°C or 29°C.

A second observable trend was that cold stress at 12°C had a greater negative impact on both germination and growth rate than heat stress at 29°C. This can be seen in Figure 3 where the 12°C plants had the longest germination time and in Figure 4 where the 12°C plants had statistically significant less growth.

A final trend, which was observed during this experiment, was that plants within each temperature treatment developed a distinct architecture. The 12°C plants, for example, had comparatively thicker leaves than the other two treatments. The 29°C plants had thinner bodies and hyponastic leaves which reached in a more upward direction than the other two treatments.



**Figure 3:** Graph of average time (days) to 100% germination for *A. thaliana* seeds at 12°C, 20°C and 29°C.



**Figure 4:** Grouped scatter plot with 95% confidence intervals for *A. thaliana* average height (mm) versus time (days) at 12°C, 20°C and 29°C.

**Sample calculation of 95% confidence interval for day 25 - 20°C treatment.**

$$\text{standard deviation } \sigma = \sqrt{\text{variance}} = \sqrt{\frac{1}{N-1} \sum_{i=1}^N (x_i - \mu)^2} \text{ where } \mu = \frac{1}{N} \sum_{i=1}^N x_i$$

$$95\% \text{ Confidence Interval: } \bar{x} \pm 1.96 \times \sigma$$

## Discussion:

This experiment investigated the impact of temperature on the germination and growth rates of *A. thaliana*. Our null hypothesis ( $H_0$ ) was that a temperature variation within the viable temperature range for *A. thaliana* would either increase or have no effect on its germination or growth rate. By the end of the experiment there was a significant difference in growth rates in the 12°C and 29°C treatments. This can be observed in the 95% confidence intervals for the mean daily height of the three treatments which do not overlap at the end of the experiment, days 19, 21 (see Figure 4). Based on this analysis, we reject the null hypothesis and support the alternate hypothesis that a temperature variation, either an increase or a decrease from an optimal

temperature within *A. thaliana*'s viable temperature range, will decrease *A. thaliana*'s growth rate.

A review of the literature helps explain why moderate changes in temperature might result in statistically significant differences in germination and growth rates. The cold stressed plants (12°C) in this experiment displayed delayed germination, reduced stature, and thicker leaves. Lee *et al.* (2005) identified over 900 genes in *A. thaliana* that changed expression when the plant was cold stressed. Many of those genes were associated with growth control chemicals, such as auxin, gibberellic acid (GA) and salicylic acid (SA). Shibasaki *et al.* (2009) investigated one of these growth control chemicals, auxin, and found that a 12 hour exposure to 4°C depressed the shootward auxin transport within *A. thaliana* by 50%. This mechanism might explain the lack of height in our cold stressed plants. Achard *et al.* (2008) proposed that cold induced C-repeat/drought-responsive element binding factor (CBF1) decreased GA synthesis. GA breaks down DELLA proteins, which repress growth. So a second reason our cold stressed plants might have less growth could be low GA concentrations that would result in an accumulation of DELLA proteins and growth repression. Scott *et al.* (2006) suggested a third possible reason for the low growth observed in the cold stressed plants. He observed high levels of cell growth depressing SA in cold stressed *A. thaliana*. Utilizing a mutant *A. thaliana* that produced an SA destroying enzyme, he found a 270% increase in biomass in the mutant when compared to the wild type. Cold-stress also could have influenced non genetic aspects of cell growth, by decreasing chemical reaction rates in the plants and decreasing cell membrane fluidity, resulting in slower membrane transport (Chinnusamy *et al.* 2007).

The results of this experiment also showed reduced growth and germination rates in the plants grown at a higher temperature. The heat stressed plants (29°C) displayed delayed

germination, elongated shape, and hyponastic or upward reaching leaves. Like cold stress, a portion of this heat stress response likely lies with altered gene expression. Heat for example, can increase auxin production in some areas of the plant, which Tao *et al.* (2008) suggested might be responsible for hyponastic leaves. Heat also increases levels of Abscisic Acid (ABA); a hormone that slows cell growth in seeds (Toh *et al.* 2008). Elevated ABA therefore may account for the delayed germination observed in the 29°C seeds. Heat stress also has been reported as interfering with photosynthesis, and to be correlated with a lower Rubisco (Ribulose 1-5 bis phosphate carboxylase) activity (Kurek *et al.* 2007). This lower activity occurs because Rubisco, an enzyme that is involved in the first step of carbon fixation, is activated by Rubisco activase that is extremely heat labile (Kurek *et al.* 2007). Lower photosynthesis rates could therefore have been part of the reason behind the lower average growth rates observed in the 29°C plants.

A major error in this experiment lies in the non-uniform illumination of the growth chambers. As the growth chambers were shared, the illumination levels were preset. As a result the 12°C growth chamber received 4900 lux, 20°C chamber received 4300 lux, and the 29°C growth chamber received 3500 lux of light. As photosynthesis rate is proportional to the number of photons received, higher light density could have resulted in a higher than expected photosynthesis rates in the 12°C growth chamber and a lower than expected photosynthesis rate in the 29°C. Other errors within this experiment could have occurred in the measuring and recording of plant growth. Since the soil at the top was not perfectly flat, there may have been some measurement error, resulting in an inaccurate data, and results. Acentric seed placement and uneven watering may have affected the growth of *A. thaliana*. Seeds near the edges of the pot may not get as much nutrients as ones in the middle, and the 29°C treatment may have had less water than others because of higher evaporation rate. Variation may also have arisen if the



nutrient content in the soil differed from pot to pot, affecting the plant growth and our results. Large genetic variability within the samples might also influence the results by giving an inaccurate representation of the effect that the temperature had on the plants' growth. Intra-chamber air borne contaminants could also have impacted the results. Otsuka *et al.* (2004) for example, found released gibberellin from one experimental group impacted other groups stored in the same growth chamber.

## **Conclusion:**

In this experiment we observed statistically significant lower growth rates in *A. thaliana* that was exposed to temperature variations (12°C and 29°C). Consequently, we rejected our null hypothesis that temperature variations increased or had no effect on the germination and growth rates of *A. thaliana* and support the alternative hypothesis that temperature variations decreased *A. thaliana*'s germination and growth rates.

## **Acknowledgements**

We would like to thank Biology 342 Professor Pollock and lab technician Mindy Chow for providing us with experimental materials and for all their support and guidance during the design and execution of this experiment. We would also like to thank our teaching assistants Niki Holden and Diana Rennison, for their suggestions and support, the herbarium staff for providing us with space to conduct our experiment, and our Biology 342 classmates for listening to our ideas and offering their suggestions. Finally, we would like to thank UBC for the opportunity to take this course.

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