The effect of temperature and the *cer10* mutation on the growth rate of *Arabidopsis thaliana*.

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Abstract

Arabidopsis thaliana is a plant species common to North America, Europe and Asia. The *cer10* mutant lacks wax structures responsible for water retention and stress tolerance. Our experiment looked to determine if temperature had differential effects on the growth of wild-type *A. thaliana,* and the *cer10* mutant. We conducted a separate treatment experiment; placing four replicates of each group (each containing three pseudoreplicates) into three separate incubators (17°C, 20°C and 25°C). We tracked growth over 15 days by measuring the change in each pseudoreplicate's primary vein length. A two-way ANOVA test was conducted upon three respective sets of hypotheses. The first test supported our alternative hypothesis that temperature has an effect on the growth of *A. thaliana (p*-value of 1.86×10^{-4}). The second test supported our alternative hypothesis that the *cer10* mutation has an effect on the growth of *A. thaliana* (*p*-value of 2.27×10^{-3}). However, we failed to reject the third null hypothesis that temperature does not have a differential effect on growth between the two species classes (*p*-value of 0.28). In conclusion, our experiment indicates that the effects of temperature are no different between wild-type and mutant *A. thaliana*, but nonetheless, both the *cer10* mutation and temperature independently have an effect on the growth of *A. thaliana.*

Introduction

Every organism has an optimal temperature range that yields the highest rate of metabolism (Gillooly *et al.* 2001). *Arabidopsis thaliana,* a small flowering plant belonging to the mustard family, is no exception. It grows natively throughout Asia, North America, and Europe, and has been observed to grow optimally in regions with environmental temperatures between 22-23°C (Meinke *et al.* 1998; Rivero *et al.* 2014). The wild type we are studying, Col-0, can be identified by the chemical profile of its epicuticular wax coating–the most apical layer of wax covering the leaves, stem, and stock. Col-0 wax is composed of primary alcohols (26, and 28 carbons in length), and alkanes (29 and 31 carbons in length) in equal amounts, whereas the *cer10* mutant is observed to have a reduced number of C29 alkanes, and an increased number of C30 primary alcohols (Rashotte *et al.* 2001). Moreover, the *cer10*

mutant is characterized by a pronounced reduction in height at roughly one-third of the Col-0 wild type, sterility under low humidity conditions due to withering of the organism, and the absence of specific fatty acid wax structures on epicuticular regions of stocks, stems, and leaves (Koornneef *et al.* 1989).

The goal of our study was to determine whether changes in environmental temperature yielded changes in the growth rate of *A. thaliana*, where growth is adequately monitored by taking incremental measurements of the leaves' primary vein length (Stewart *et al.* 2016). Secondly, we assessed whether changes in environmental temperature had differential effects on growth rates of wild-type *A. thaliana,* and the *cer10* mutant *A. thaliana.* These objectives led to three sets of hypotheses:

H_{01}: Environmental temperature has no effect on the growth rate of *A. thaliana*.

H_{A1}: Environmental temperature has an effect on the growth rate of *A. thaliana*.

H_{02}: Presence of the *cer10* mutation has no effect on the growth rate of *A. thaliana*.

H_{A2}: Presence of the *cer10* mutation has an effect on the

H_{03}: The effect of environmental temperature does not have differential effects on the growth rate of the Col-0 wild-type *A. thaliana* and the *cer10* mutant *A. thaliana*

H_{A3}: The effect of environmental temperature has a differential effect on the growth rate of the Col-0 wild-type *A. thaliana* and the *cer10* mutant *A. thaliana.* Within the scope of climate change, the conclusions drawn from our hypothesis testing have important implications for the survival and distribution of *A. thaliana*. The Intergovernmental Panel on Climate Change (2007) has projected that within the 21st century, we can expect to see a 1-4°C increase in global surface temperature. If this projected increase occurs, we may see a shift in the distribution of *A. thaliana* poleward. This is because polar regions with previously sub-optimal temperatures will be augmented towards the optimal range, yielding greater growth and higher abundance of the species in these areas. Conversely, regions near the

equator with surface temperatures that were previously above-optimal will be increased further, potentially into a range that is no longer tolerable, and therefore cause a reduction in the species abundance. This poleward progression of distribution range was previously seen for *A. thaliana* following its postglacial recolonization of Eurasia, and can be considered analogous to current and future conditions (Sharbel *et al.* 2000).

We predicted that temperature would have an effect on the growth rate of both mutant and wild-type *A. thaliana,* and that this effect would be more pronounced in the *cer10* mutant. Furthermore, we predict that the *cer10* mutation will have an effect on growth, independent of temperature. Our prediction was based on a study conducted by Zheng *et al.* (2005) which indicated that the *cer10* mutation resulted in a reduction of very-long-chain fatty acid (VLCFA) synthesis. These compounds are primary components of the organism's epicuticular wax coating which, as a whole, is responsible for stress tolerance and moisture retention in the leaves of the organism (Jenks *et al.* 1995). As per the reduction of VLCFAs, we postulate that at higher temperatures we will observe a greater decline in photosynthetic efficiency, as water is a key reactant in photosynthesis (Figure 1). We believe that this will result in a lower growth rate of the *cer10* mutant when compared to the wild-type *A. thaliana.*

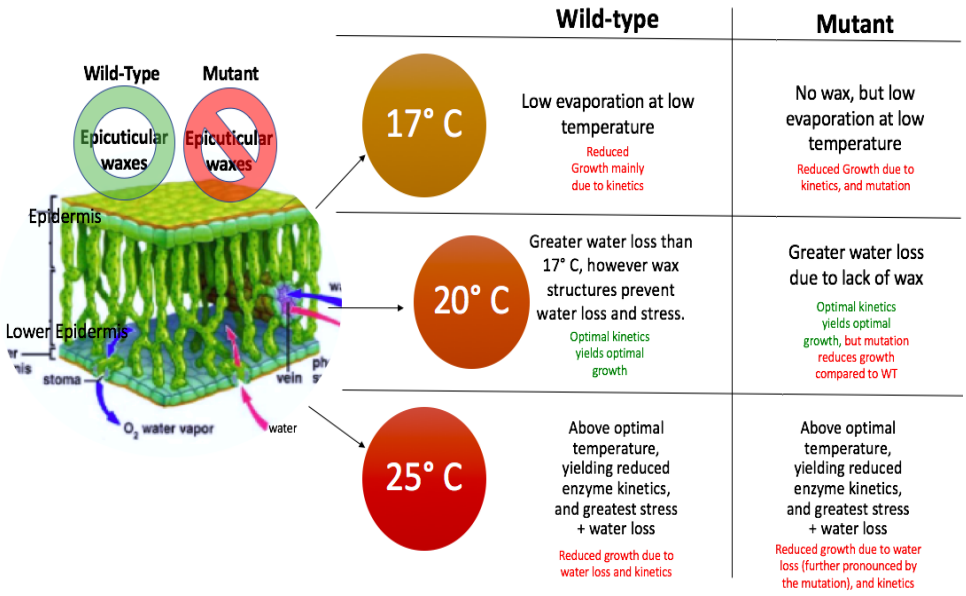

Figure 1. A biological model summarizing our predictions and their causes.

Methods

We conducted our experiment over 15 days, which included planting seedlings to taking final plant measurements. We designated eight pots for each treatment (17°C, 20°C and 25°C), with four pots for the wild types and four pots for the mutants. We selected the 20°C treatment as the control treatment because it was the closest optimal temperature for *A. thaliana* seedling growth (Rivero *et al.* 2014). The seedlings were grown 10 days in advance. When transplanting, we transferred three pseudoreplicate seedlings into one pot and set color-coded toothpicks near each pseudoreplicate (Figure 2). We took measurements from the leaf which was closest to the toothpick for consistency.

Figure 2. Color coded toothpicks indicate which leaf we should measure.

After planting, we measured the initial primary vein length (Figure 2) of each seedling and maintained soil saturation by adding 600 mL, 900 mL and 1200 mL of water into the large plastic trays which held all the pots for different treatments, with more water at higher temperatures. Lastly, we put different treatments into the corresponding incubators. We measured the light intensity inside each incubator using a luxmeter and put cheesecloth above our 17°C and 25°C treatments (Figure 4) in order to control for light intensity. We kept the light intensity differences between treatments within 200 lux.

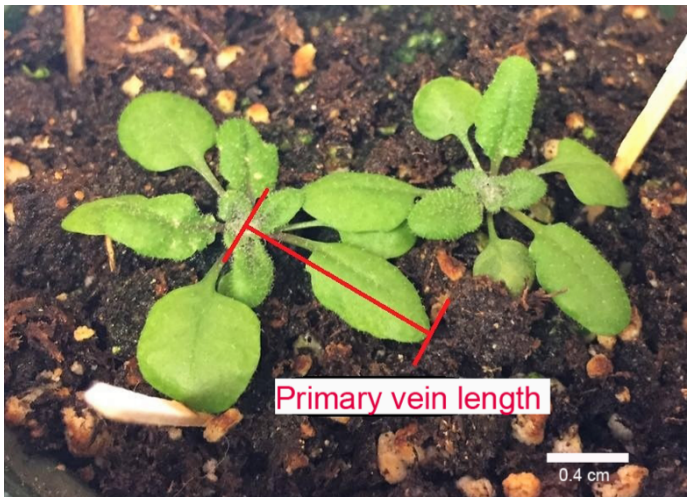

Figure 3. How we measured primary vein length of *A. thaliana*.

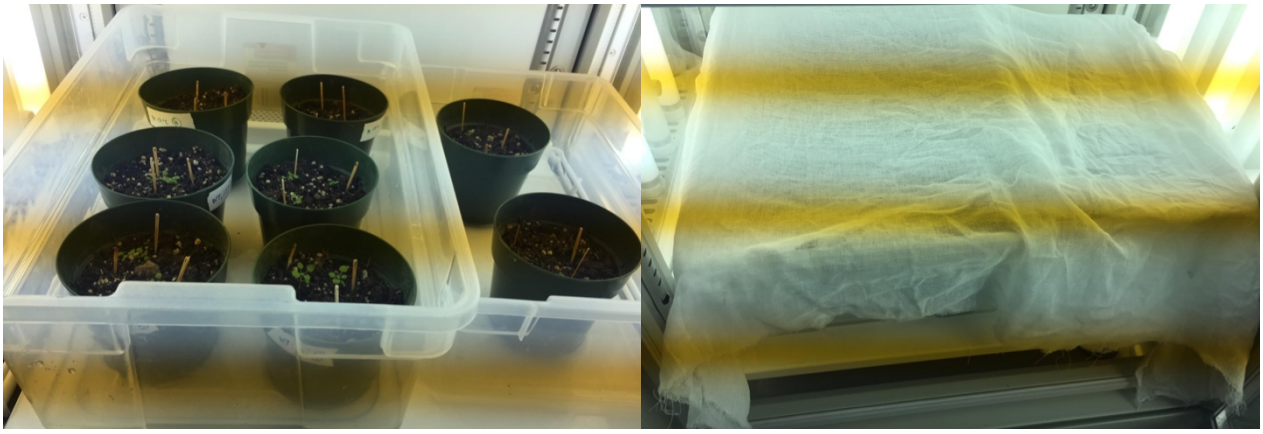

Figure 4. 17°C treatment in the incubator and covered with cheesecloth.

We took measurements on day 1, day 3, day 7, day 9, day 10 and day 14. The time period that the treatments spent out of the incubators each day was consistently 30 minutes amongst all the treatments. After the measurements were complete, we replenished all pots to their initial volumes of water and put them back into the incubators. These steps were repeated during each measurement period.

In order to analyze our data, we took the mean primary vein lengths. This was graphed against time, and a linear plot was constructed to determine primary vein growth rate (indicate by the slope of the line). This was done for all replicates across all treatments. We calculated the mean primary vein growth rate and 95% confidence intervals and used a two-

way ANOVA statistical analysis to calculate our *p*-values and variation seen from our confidence intervals are indicated on our graphs.

Results

Considering the combined (wild type and mutant) mean primary vein growth rate from day 7 to day 14, the fastest mean growth rate was observed at 20°C, (0.13 ± 0.05 cm/day) while the slowest was at 17°C (0.02 ± 0.01 cm/day). The mean growth rate at 25°C was 0.09 ± 0.04 cm/day. We calculated a *p*-value of 1.86×10^{-4} associated with hypothesis one.

Without taking into consideration temperature, the wild-type *A. thaliana* grew at a faster rate (0.11 ± 0.05 cm/day) compared to the mutant (0.05 ± 0.02 cm/day). A *p*-value of 2.27×10^{-3} was calculated for hypothesis two.

Figure 5 shows the differential effects of temperature on the mean primary vein growth rate of the wild-type and mutant *A. thaliana*. This is investigated in hypothesis three which has a calculated *p*-value of 0.28. For each of the three temperature treatments, the wild-type plants grew at a faster mean rate in comparison to the mutant plants. The wild-type plants at 17°C had a mean primary vein growth rate of 0.03 ± 0.01 cm/day, at 20°C a mean of 0.18 ± 0.07 cm/day, and at 25°C a mean of 0.12 ± 0.05 cm/day. For the mutant plants, a mean of 0.01 ± 0.02 cm/day was observed at 17°C, 0.09 ± 0.02 cm/day at 20°C, and 0.05 ± 0.04 cm/day at 25°C. However, only in the 20°C case is there a difference in the 95% confidence intervals for wild type and mutant as they fail to overlap. The overall growth pattern of the *A. thaliana* plants in each temperature treatment is the same in wild-type and mutant plants. The 17°C treatment has the lowest mean growth rate of its primary vein contrasting with an optimal growth rate at 20°C. The 25°C treatment shows growth rates at the middle of the two (Figure 5).

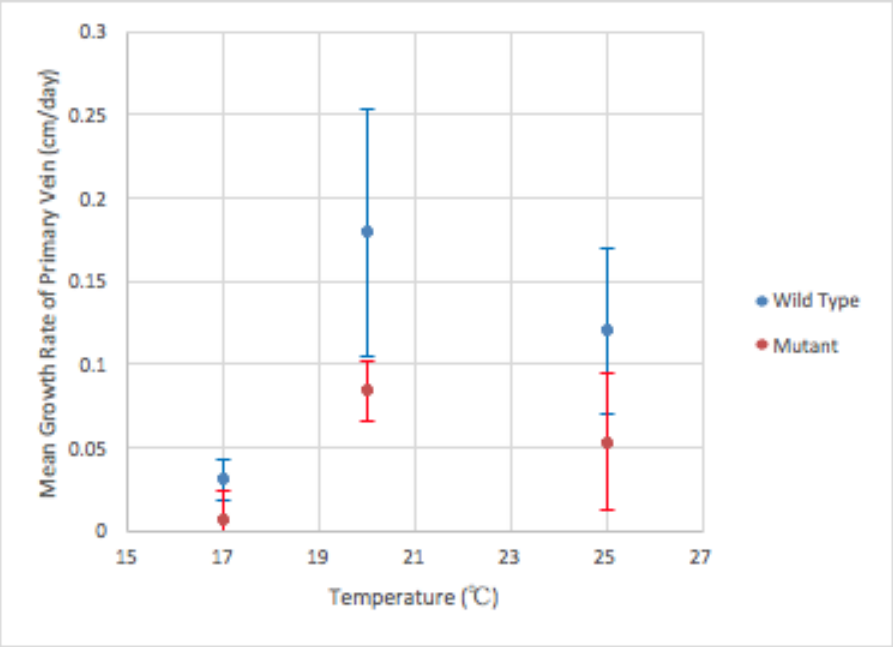

Figure 5. The mean primary vein growth rate of wild-type and mutant *A. thaliana* from day 7 to day 14 at 17°C, 20°C, and 25°C. Bars represent 95% confidence intervals, n=4, p=0.28.

Qualitatively, there were obvious differences between wild-type and mutant plants for all temperature treatments. Mutant leaves appeared to be darker, more wrinkled and curled compared to wild-type leaves. Additionally, wild-type plants grew upward and larger in size. Overall, the wild-type plant had the highest maximum leaf number per plant with nine while mutant plants had seven leaves as their maximum. The leaves on both classes of plants showed evident trichomes, but a greater number were observed on the wild-type *A. thaliana.*

The leaves grown at 17°C and 25°C were smaller compared to those at 20°C but had longer stems. Plants at 17°C had stems longer than that of 20°C but shorter than that of 25°C. The mutant plants had a higher number of dead pseudoreplicates at 18 across all three treatments, compared to the two for wild-type plants.

Discussion

Based on the p-values obtained from the two-way ANOVA, we reject our null hypotheses H_{01} and H_{02}, (p values are less than 0.05) and provide support for our alternate

hypotheses H_{A1} and H_{A2}, supporting our prediction. We fail to reject our null hypothesis H_{03} as the *p*-value is greater than 0.05.

Hypothesis 1

A *p*-value of 1.86×10^{-4} indicates that temperature has an effect on the growth rate of *A. thaliana*, agreeing with our prediction. This is consistent with the findings of Rivero *et al.* (2014) who state that the optimal growth is between 22-23°C.

Lower temperatures had a consistently more dramatic effect than high temperatures on growth rate. We propose that the statistically significant difference in growth at 17°C is due to temperature's effect on slowing down the rate of metabolic reactions (Gillooly *et al.* 2001). Meanwhile, 25°C is not far from the plant's optimal range, so the effect is not as pronounced, though still evident.

Stewart *et al*. (2015) noted that *A. thaliana* planted in lower temperatures had thicker leaves, suggesting that chloroplasts were stacked vertically to increase the photosynthesis-to-area ratio. This reduces the energy spent on growing biomass, and increases the storage of energy for the expected winter. This may be a possible explanation as to why the leaf stem and leaf size was smaller.

Hypothesis 2

Although the confidence intervals overlap in the 17°C and 25°C treatments, a *p*-value of 2.27×10^{-3} indicates that the presence of the *cer10* gene mutation has a significant effect on the growth rate of *A. thaliana*. Jenks *et al*. (1995) established the importance of the epicuticular wax layer on stress tolerance. We suggest that the presence of the wax layer in the wild type contributed to overall fitness by allowing the plants to withstand temperatures outside their optimal range. This is further supported in our study by noting the much greater number of dead mutant pseudoreplicates compared to the wild type.

Trichomes are highly specialized unicellular structures that serve to control temperature, regulate water, and act as barriers to protect plants from herbivores, parasites and UV irradiation. (Gutierrez-Alcala *et al.* 2000; Maes *et al.* 2008; Yu *et al.* 2010). Telfer *et al.* (1997) proposed that the abundance and distribution of trichomes on *A. thaliana* is temporally regulated. The higher abundance of trichomes found on the wild type may be an indication of reproductive maturity (Larkin *et al.* 1996). Conversely, the fewer trichomes on the mutant may suggest that it is not yet approaching reproductive maturity, thus is growing slower.

Hypothesis 3

With a *p*-value of 0.28, we do not have enough support to state that the effect of temperature leads to significant differences in growth rate pattern between wild-type and mutant plants. This is in contrast to our initial prediction that a difference would be seen due to the mutant's inability to develop tolerance to harmful temperatures as a result of the lack of the epicuticular wax layer (Hong & Vierling 2000). Although our results showed that the wild-type plants had a higher mean growth rate for each treatment, the pattern of growth rate for wild type and mutant seen across temperatures cannot be said to differ. The choice of temperature conditions and experimental setup could suggest why results were not statistically significant. The consequence of the missing epicuticular wax layer may be more pronounced if we tested for more extreme temperature ranges, above the optimal 22-23°C (Rivero *et al.* 2014), or if we did not keep the soil saturated with water. Since increasing temperature requires plants to uptake more water, the lack of wax layer contributing to the lack of water retention (Shepherd & Griffiths 2006) would affect the growth of the plant. By keeping the soil saturated with water, the differences between the wild type and mutant's ability to retain water became irrelevant, and may not impose any statistically significant differences on their growth rates.

Uncertainty and variation

In our experiment, the systematic errors can come from measurements and instrument drifts. The leaves we measured were as small as 0.08 cm. Especially for the curved mutant leaves, the readings from calipers varied. We used tweezers to unfold the leaves but that can create uncertainties as the leaves were still slightly curled. We took the mean of three pseudoreplicates to reduce errors. For instrument drift, the only device that we used was a light meter. This systematic error does not affect our results too much because we kept the light intensity consistent with the difference less than 200 lux

Random error in our experiment resulted from natural biological variation. We cannot control the natural biological conditions of the seedlings, therefore we took means from three pseudoreplicates to minimize variation among replicates. Pseudoreplicates began to die as time went on. The reason for their death can be varied, such as improper rooting when we planted them or they succumbed to the temperature conditions. This random error definitely can affect our results with lower growth rate. We removed all the data of dead pseudoreplicates from our analysis because the dying plants skewed the growth rate data to be lower than living plants. Ultimately, there was always at least one pseudoreplicate alive in each replicate for us to use as a data point.

Conclusion

Based on our results, temperature had an effect on the growth rate of *A. thaliana*. Additionally, the presence of the *cer10* mutation had an effect on the growth rate of *A. thaliana*. There was no differential effect of temperature on the growth rate of wild-type and mutant plants. The first two results agree with our prediction– however, the final fails to do so. Conducting this research is important in understanding the biological distribution of plants, such as *A. thaliana,* as they are subjected to the environmental stresses of temperature.

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