UNIVERSITY OF GOTHENBURG

DEPARTMENT OF BIOLOGICAL AND ENVIRONMENTAL SCIENCES

# Acclimation of photosynthetic heat tolerance in a tropical species *Harungana montana* grown at different temperatures

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Photosynthetic heat tolerance measurement: leaf-clip in closed position in a leaf from *Harungana Montana*. Picture taken by Claire Ract.

**Abstract**

In a global warming context, plants are constantly exposed to extreme temperatures which might lead to irreversible deteriorations of the photosystem II (PSII) and therefore on the whole photosynthetic mechanism. In tropical regions where temperatures are in average already high, it is common for plants species to encounter high temperatures. However, only a few studies have been performed on thermal tolerance of tropical species. For this project, emphasis was on heat tolerance of a tropical tree species *Harungana montana*. This species was grown at three different temperatures (20, 25 and 30°C, six plants in each temperature treatment) under controlled conditions in growth chambers. The impact of growth temperature on photosynthetic heat tolerance was tested by inducing a range of ten different temperatures to the leaf from 20°C to 54°C. Two indicators of heat tolerance were determined; i) the critical temperature ($T_{crit}$, at which the photosystem II starts to be damaged), and ii) $T_{50}$, the temperature at which the quantum yield of the photosystem II has reduced to 50% compared to unstressed leaves. The results showed that $T_{crit}$ and $T_{50}$ were greater for individuals acclimated to higher growing temperatures. However, their acclimation was less than the rising of temperatures between the treatments. This indicates that plants will not be able to keep pace with increasing temperatures ($T_{crit}$ was 4°C higher in 30°C than 20°C plants and $T_{50}$ was 2.5°C higher). $T_{crit}$ was related to differences in membrane lipid composition and in leaf structure. Higher $T_{crit}$ was positively correlated to a greater amount of saturated fatty acids, (to maintain membrane stability at higher temperatures) and negatively correlated with a lower leaf mass per area. These results thus show that plants grown at high temperatures acclimate structurally and physiologically to better tolerate extreme leaf temperatures. However, above a certain upper limit, irreversible negative effects will occur damaging the plants.

**Key words:** acclimation, chlorophyll fluorescence, critical temperature, fatty acids, Leaf mass per area, photosynthetic heat tolerance, quantum yield of photosystem II.

## I. Introduction:

Due to the global warming, nowadays, high temperatures and extreme heat waves are omnipresent. Yet, those high temperatures impact a lot of living organisms on earth. As plants are immobile and therefore cannot migrate, they can be more affected compared to others (Curtis et al., 2014). It has been shown that extreme temperatures might constrain the plant's metabolic process as photosynthesis for example as well as leads to photosystem damage (Zhu et al., 2017; Marutani et al., 2012). Yet, it is predicted that the frequency and severity of those high heat waves are likely to increase in a near future. Recent studies demonstrated that tropical species might be specifically affected as they are already growing near their maximum absolute temperature range. However, we do not have a lot of knowledge about the thermal tolerance and the maximum temperature that tropical species can support (Sastry and Barua, 2017). In order to anticipate the reaction of plants species to the changing climatic conditions, we have to know how they tolerate heat and how they respond to it (Curtis et al., 2014).

The photosystem II (PSII) is a protein complex located in the thylakoid membrane of chloroplasts. Together with the photosystem I (PSI), they are performing photosynthesis by absorbing the light and driving the photosynthetic electrons transport. Nevertheless, PSII is believe to be the most vulnerable site in case of stress due to extreme heat (Curtis et al., 2014). In point of fact, in that case, the activity of PSII will follow a decline leading to several damages in the thylakoid membrane. Therefore, higher temperatures can reduce a lot of the plant's functions as photosynthesis, growth rate or the activity of photosystem II (PSII) at the leaf level (Teskey et al., 2015). By contrast, PSI is less sensitive to high temperatures (Krause et al., 2010).

The method commonly used for determining the upper temperature where the heated leaves started to be damaged is called chlorophyll fluorescence. It is a measure of the light re-emitted by PSII, providing a measure of the functioning of photosynthesis as well as plant's response to environmental change (Murchie and Lawson, 2013). For these reasons it is a very advantageous technique to measure the short-term effects of heating and their damages caused to photosynthetic tissue by evaluating the activity of PSII in plants. Fluorescence gives information of two processes that are especially important for understanding heat tolerance, the quantum yield of PSII and the critical temperature. The performance of PSII is given by the ratio $F_V/F_m$. Here $F_V$, called the variable fluorescence is determined by doing $F_m$-$F_{0,}$ with $F_m$ the maximal level of fluorescence and $F_0$, the minimal level of fluorescence. It permits to know in this study, if the leaves were stress due to heat. In fact, if it results in an amount lower than 0.83, the leaves are under some types of biotic or abiotic stress which reduce the capacity for photochemical process and lead to damages of the PSII. If the leaves are not stressed, the value of the ratio will be 0.83, and it corresponds to the maximum quantum yield of photosynthesis (Murchie and Lawson, 2013). In this study I have focussed on $T_{50}$, the temperature where the quantum yield had reduced to 50% compared to unstressed leaves at low temperature.

The quantification of photosynthetic heat tolerance is done by measuring the critical temperature ($T_{crit}$), which is the temperature at which the minimal level of fluorescence of leaves increases sharply. This temperature reflects the upper threshold above which normal functions of PSII start to be damaged due to heat (Zhu et al., 2017).

Previous studies showed that, $T_{50}$ was higher for plants species growing under higher temperatures (in the desert) compared to others species growing at costal field sites (increasing of 0.6°C per 1°C rising of habitat temperature) (Knight and Ackerly, 2002). Similarly, an early study done on seasonal variation of $T_{crit}$ showed that its higher values was display by plants coming from hotter sites (Zhu et al., 2017).

Two common types of temperature acclimation of the plant leaves are: i) leaf structure and ii) adjustment in membrane lipid/fatty acid composition. The first one was the leaf dry mass per area (LMA). This parameter is positively correlated with the leaf density (LD) which explained 80% of the variation of LMA and the leaf volume per area (LVA) which explained 20% of the variation in a global meta-analysis (Poorter et al., 2009). Therefore, as the result of a decline in LMA, its density and volume will decrease as well. Consequently, the leaf internal structure will be constituted of a great volume of air spaces. This structure will enable the leaf to better transfer the heat passing through it (Poorter et al., 2009). Therefore, a low LMA value enable the leaf to reduce the likelihood of high temperatures with a particular leaf structure and facilitate the heat to move away

effectively. This is a type of structural acclimation to high temperatures by the avoidance of heat and thus, reduce the need for higher $T_{crit}$.
The second one happens at the level of the leaf physiology. A higher level of saturation of fatty acids (FA) in the lipid bilayer of cell membrane will cause an amplification of membrane permeability and flexibility. Thanks to this modification of the membrane network, it will cause a decline of the photosynthetic electron transport activity and an inhibition of photosynthesis (Hüve et al., 2011; Teskey et al., 2015). Due to the physical properties of lipids, plants can adapt to higher temperatures by the addition of FA highly saturated characterize by a high level of saturated acyl chains. Those modifications will have a high impact on the thermal stability of photochemical process (the photosynthesis) and will be related to a rise of thermal tolerance and therefore, to $T_{crit}$ (Zhu et al., 2017).

In this project, we studied three fatty acids class: MGDG (monogalactosyldiacylglycerol), DGDG (digalactosyldiacylglycerol) and PG (phosphatidylglycerol). Those fatty acids class are common components of the photosynthetic membrane of plants. They are especially linked to the protein complex of the PSII (Boudière et al., 2013). The level of saturation was determined by DBI, the Double Bond Index, which is the average number of double bonds present in a fatty acid class. A fatty acid highly saturated have a low amount of double bonds.

## II. Aims of the study and hypothesis:

In this study, I investigated the effect of growth temperature on the heat tolerance of the tropical tree species *Harungana montana*. The chlorophyll fluorescence method was used to determine both $F_0$ rise and $F_V/F_m$ decline after heat pulse on the leaf. I hypothesized that:

i. Individuals acclimated to warm conditions will show signs of greater quantum yield (higher $F_V/F_m$ ratio and therefore higher $T_{50}$ value) at high temperatures than the individuals acclimated to cooler conditions.

ii. Critical temperature, $T_{crit}$, acclimates to changes in growth temperature and its value will be higher in individuals acclimated to warm conditions than in individuals living under cooler conditions.

iii. Plants adapted to high temperatures display a low leaf mass per area (LMA) because a low leaf density leads to more efficient transfer of heat away from the leaf.

iv. Individuals acclimated to higher temperatures exhibit a higher saturation level of leaf membrane fatty acids, which allows them to better maintain the membrane stability under extreme heat compared to plants grown in lower temperatures.

## III. Materials and methods:

### Plant material

This study was done on *Harungana montana*, a tropical tree species belonging to the Hypericaceae family. The studied plants were grown from seeds originating in Rwanda, central Africa. This particular species was chosen in this project because it has been studied in Rwanda before without giving relevant results (probably due to a lot of variations in the field). Therefore, we decided to study this species in controlled conditions to see if it can affect the results. The plants were grown in growth chambers under controlled climatic conditions. Three temperature treatments were applied with six plants growing temperature as well as the quantity of carbon dioxide was controlled. The plants had grown for two years in the botanical building of the University of Gothenburg. There were 18 plants in total, 6 per growing temperatures, which were the following: 20, 25 and 30 degree Celsius (daytime temperatures). During night time, these temperatures were 5°C cooler. We used those three growing temperatures because they do roughly cover the range of temperatures among the sites studied in Rwanda. The plants were watered three times per week and given nutrients once per week.

### Chlorophyll fluorescence

The photosynthetic heat tolerance was determined by chlorophyll fluorescence on one leaf per plant using a Pocket-PEA fluorimeter (Hansateck, King's Lynn, England). The instrument was configured to measure during 1 second at the highest light intensity (3500µ mol $m^{-2}$ $s^{-1}$). The leaves were dark-adapted for at least 30 minutes before each measurement to ensure that all the reactions centres were open and fully oxidised. To assess the effect of temperature on the fluorescence parameters, $F_V/F_m$ and $T_{crit}$ , each leaf was measured at 20°C, 30°C, 35°C, 40°C, 43°C, 45°C, 48°C, 50°C, 52°C, 54°C. Leaf temperature was controlled by infrared heating lamps and measurements were made when temperature varied no more than 1 degree Celsius in 2 minutes. The range of temperatures was selected based on a previous field-based study on this species (Tarvainen et al., unpublished). Therefore, we wanted to picked a range around these values, with a high enough temperature in order to induce a certain heat damage to the leaf.

Then the temperature was put until an equilibrium, the ratio $F_V/F_m$ was measured using the fluorimeter which induces a high intensity pulse (saturating) to the dark-adapted leaf leading to a maximum value of fluorescence $F_m$. Starting from the smallest temperature, two leaves per plants were measured at the same time. Once six samples were done (for one temperature treatment), I switched to the following upper temperature.

The impact of recovery time between measurements of chlorophyll fluorescence was tested in order to justify that 30 minutes of dark-adaptation was enough for the leaf to recover from the pulse. For doing this, two plants from each temperature treatment had been chosen randomly. On each plant, 6 leaves picked randomly had been measured. The first step was to dark adapted all

the leaves for 30 minutes and then a saturating pulse was induced by the fluorimeter, as above. Following this, each of the 6 leaves had undergo another pulse after the time of dark-adaptation desired (5 min on one leaf, 10 min on another one and following: 15, 20, 25 and 30 minutes). The measurements were done at room temperature. After this step, the percentage of difference between the initial $F_V/F_m$ value (measured after 30 minutes) and the final ratio value obtained after the exclusive recovery time (5, 10 minutes…) was calculated.

## Data analysis

For the analysis of the quantum yield of PSII, the ratio $F_V/F_m$ was normalised to range from 0 to 1 and plotted against measurement temperature. The temperature responses were fitted with the non-linear Gompertz function for each temperature treatment:

y= a*exp(-b*exp(c*temperature)). Here, y is half of the maximum value of the ratio (after normalization should be 0.5), a is the maximum value of the ratio (should be close to 0.83, therefore 1 after normalization), b correspond to the shift of the curve along the x-axis and c is the rate of decay of the ratio with temperature.

The aim of doing this was to find $T_{50}$, the temperature where $F_V/F_m$ for unstressed leaves at low temperature had reduced to 50% (from its initial value: 0.83). Another value which was determined is $T_{80}$, the temperature where the ratio has reduced to 80 percent. The reason to include this parameter was that since high temperatures are still fairly uncommon $T_{80}$ gives perhaps a better indication of the type of heat stress plants have to endure regularly in the field in Rwanda. Whereas $T_{50}$ indicated quite uncommon severe stress conditions, but it's useful as a reference value for comparing our findings to other studies that mostly report $T_{50}$. Using the Gompertz model, the values of both $T_{50}$ and $T_{80}$ were determined thanks to its function: temperature here is the value we wanted to determine: $T_{50}$ or $T_{80}$. After isolation of temperature, we found: $T_{50}$ =(ln(ln(2)/b))/c and $T_{80}$=(ln(ln1,25)/b))/c.

The value of minimal fluorescence, $F_0$ was used to quantify the photosynthetic heat tolerance, using the critical temperature, $T_{crit}$. This parameter was calculated at the intersection of two regression lines (portions of slow and rapid fluorescence) using segmented piecewise linear regression model which fitted both lines at the same time. We calculated $T_{crit}$ using both equations: y1 = ax + b and y2 = cx + d, with a and c the slopes, b and d the intercepts and x corresponding to $T_{crit}$, the value we are interested in. To determine this value, we did y1=y2, given x = ((d-b)/(a-c)).

The Solver Microsoft Excel add-in program was used to maximize the R2 value (closer to 1) which determine how closely the data are to the fitting (Gompertz model and segmented regression line), allowing to obtain an optimal value of $T_{50}$, $T_{80}$ and $T_{crit}$

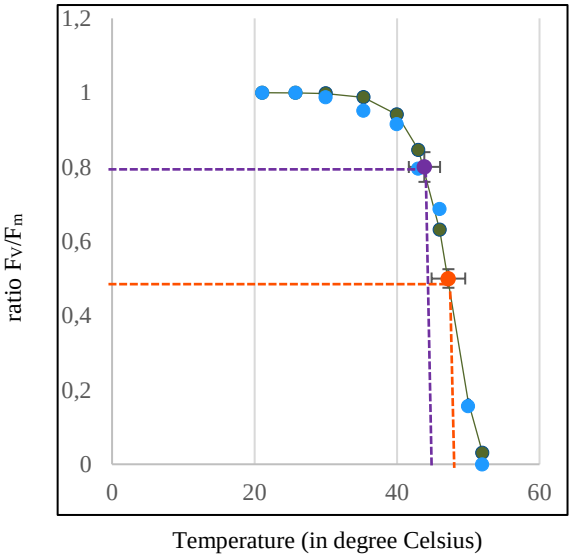


Figure 1) a: Example of computation of $T_{50}$ and $T_{80}$ among one leaf: D20 which had grown at 20 degree Celsius. The blue circles correspond to the observed values of the ratio $F_V/F_m$ normalized. The dark green line is the Gompertz model used in order to easily determine $T_{50}$ and $T_{80}$. The orange dotted line represents $T_{50}$ and the purple one is $T_{80}$.

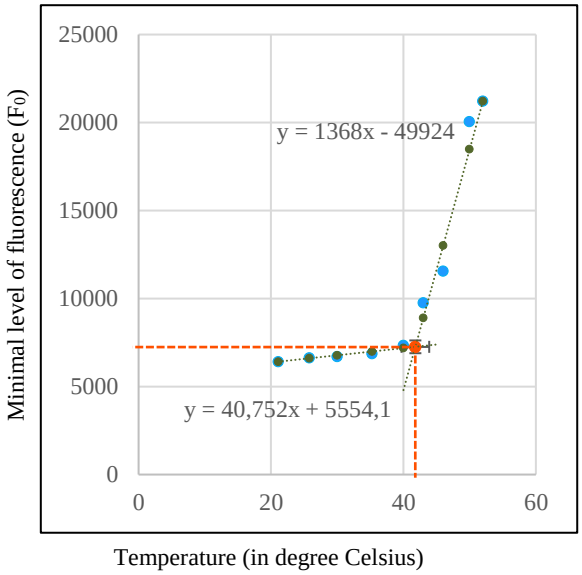


Figure 2) b: Example of the computation of the critical temperature for one leaf: D20 which have grew at 20 degree. The blue circle corresponds to the value of $F_0$ observed. The dark green circles and trend lines represent the segmented piecewise linear regression model used to determine $T_{crit}$ at the intersection of both trend line of the model. The orange dotted line is $T_{crit}$.

## Leaf mass per area (LMA)

Leaf mass per area were determined from discs (20 mm diameter) taken from the neighbouring leaf of the one measured avoiding major veins. Those two pieces of leaf were placed in an aluminium box and were oven-dried for 3 days at 70 degree Celsius. LMA (in $g.m^{-2}$) was calculated as the ratio between the leaf dry mass in gram and the leaf area.

## Fatty acid composition analysis

The fatty acid analysis was performed by doing two holes on the neighbouring leaf of the one measured using a hole punch. Those two pieces of leaf were put inside a closed aluminium bag. Then they were freezing in nitrogen for a few minutes. The aluminium bags with the leaves were put in a freezer for 3 days at -70 degree Celsius. After having doing that, we started the extraction of polar lipids from the leaf. To perform this, a fixed volume (10 µl) of internal standard mix (containing 1 di17:0-PC and 1 µg of di17:0-PG) was added in each of the 18 glass tubes (one for each leaf disc). After adding this mix, 2ml of 2-propanol heated at 100°C had been transferred in each tube in order to dissolve non-polar compounds (isopropanol act as a solvent). The leaf discs were then fully submerged into each tube and heat for around 5 minutes. After that step, we had dried the samples until no visible fatty acid remains using nitrogen for around 30 minutes. A fixed volume (1.5mL) of a so called “one phase” solution (containing 100 ml CHCl3 ,200 ml MeOH and 80 ml H20 + 0.05% Injection 2µl) is added to homogenize the leaf discs, before closing the tubes and extract them in ultrasonic bath for 30 minutes until the leaf discs are completely

depigmented. We left the tubes for an additional 20 or 60 minutes (could be longer) in 4°C. If the discs are not depigmented yet, the samples were left at -20°C overnight. Phase separation was then induced using a fixed volume (375 µl) of chloroform (with 0.025% BHT, a phenolic antioxidant used to preserve fats) and 375µl of 2% acetic acid. The most suitable way to assist phase separation is to vortex. Therefore, we separated the homogenate into two layers: an upper, methanolic layer (water and methanol) constituted of the polar molecules, salts, amino acids, etc and a lower, chloroform layer constituted of all the fatty acids. We then transferred this lower phase to a new tube using a glass pipette. To be sure to collect all the lipids we added again chloroform (750µl with 0.025% BHT) to the original tube and we vortex and waited until the phase separation. Once this was done, we added the second lower phase into the new tubes and dried this chloroform phase using nitrogen. The final step was to dissolve the sample using 50 µl of methanol. All the mixture was then transfer to LC-vials. Following this, the detection of the lipids was done using lipid chromatography and mass spectrometry. The three principal fatty acid class were analysed and the degree of saturation was determined using the average number of doubles bonds for class. In order to determine if the fatty acid saturation level was related to the critical temperature, I plotted $T_{crit}$ against DBI (Double Bond Index) for each of the three fatty acids

### Statistical analysis

A comparison of the $T_{50,}$ $T_{80,}$ $T_{crit}$, LMA and DBI among individuals acclimated to different growth temperatures had been performed using one-way analysis of variance (ANOVA) in R studio.

Following this, a post hoc test, Tukey HSD was performed in order to determine specifically, which temperature treatments was different from one another. This stage was performed to find an evidence to our hypothesis of temperature growth acclimation.

## IV. Results:

### *Impact of recovery time of chlorophyll fluorescence measurements*

From the method used above, we had determined an average of the percentage difference between the initial and final $F_V/F_m$ ratio for the three treatments. The results showed that after 5 minutes of dark-adaptation, the difference was the greatest compared to the other dark-adaptation times. That means that the leaf did not have enough time to recover from the pulse. As seen above this difference was not very high but as the experiment was done at room temperature, the leaf did not undergo damage due to extreme heat waves, therefore, their recovery times were short. From 25 minutes, in average for the 3 growing temperatures, there were no more differences between the initial and final value of the ratio meaning, the leaves had completely recovered. In conclusion, the time of dark-adaptation that we had picked (30 minutes) was adequate for the leaves to recover.

| Time of dark-adaptation chose (in min) | Average percentage difference between the two ratios |
|---|---|
| 5 | 1.167 |
| 10 | 0.636 |
| 15 | 0.44 |
| 20 | 0.200 |
| 25 | 0 |
| 30 | 0 |

*Table 1:* Mean of the percentage difference between the initial and final $F_V/F_m$ ratio across the three temperature treatments as function as the chosen dark-adaptation times.

### *Temperature acclimation of PSII quantum yield*

The plants had suffered heat damages at different rates depending on which temperature they had grown. Figure 2 shows the PSII functioning: at lowest temperature, PSII is at its maximum capacity, whereas as temperatures increases its performance decrease. Starting at room temperature, all the leaves were unstressed and consequently the value of $F_V/F_m$ ratio was approximately around 0.83 for all the temperature treatments which correspond to the maximum quantum yield of photosynthesis. As temperature increased, the leaves undergo more damages due to heat and the value of the ratio decreased until a minimum value of the ratio is reached at the highest temperature for each treatment. From 40°C, the highest growing temperature started to have a better tolerance to heat compared to the two others. Similarly, the plants growing at 25°C better tolerated heat than the ones growing at 20°C. A One-way analysis of variance (ANOVA) was conducted, and we observed a significant difference of $T_{50}$ among the three treatments [F (2, 15) =54,92, p<0.001]. Following this, an ANOVA post hoc tests: Tukey HSD (p<0.05) was performed. The results indicated a significant difference in $T_{50}$ among the treatments at 20 and 30 degree Celsius ([F (2,15) =54.92, p<0.001]) as well as between the one at 25 and 30 degree [F (2, 15) =54.92, p<0.001]. Equivalently, a significant difference had been detected between 20 and 25°C with ([F (2,15) =54.92, p<0.05]). The same test was performed for $T_{80}$. ANOVA indicated also a significant difference among the three treatments [F (2, 15) =6.921, p<0.01]. The Tukey HSD test, demonstrated as for $T_{50}$, a significant difference among the treatment at 20 and 30 degree ([F (2, 15) =6.921, p<0.01]) as well as between those at 25 and 30 degree ([F (2, 15) =6.921, p<0.05]). However, no significant differences had been observed between 20 and 25°C.

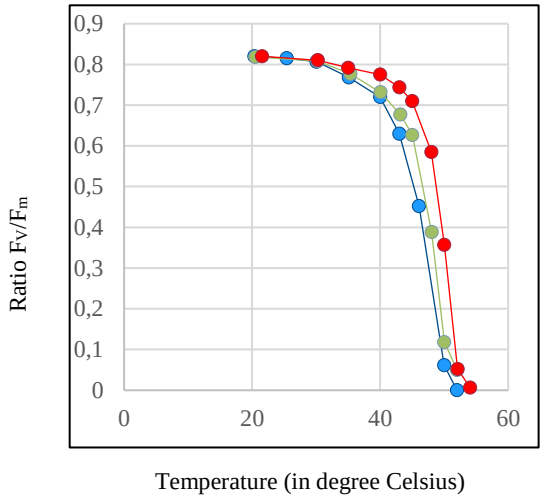


Figure 2: Comparison of heat tolerance acclimation of *Harungana Montana* at the three different growing temperatures: 20, 25 and 30 degree Celsius. Blue circles, represent the growth at 20 degree, the green circles at 25 degree and the red circles correspond to 30 degree Celsius.

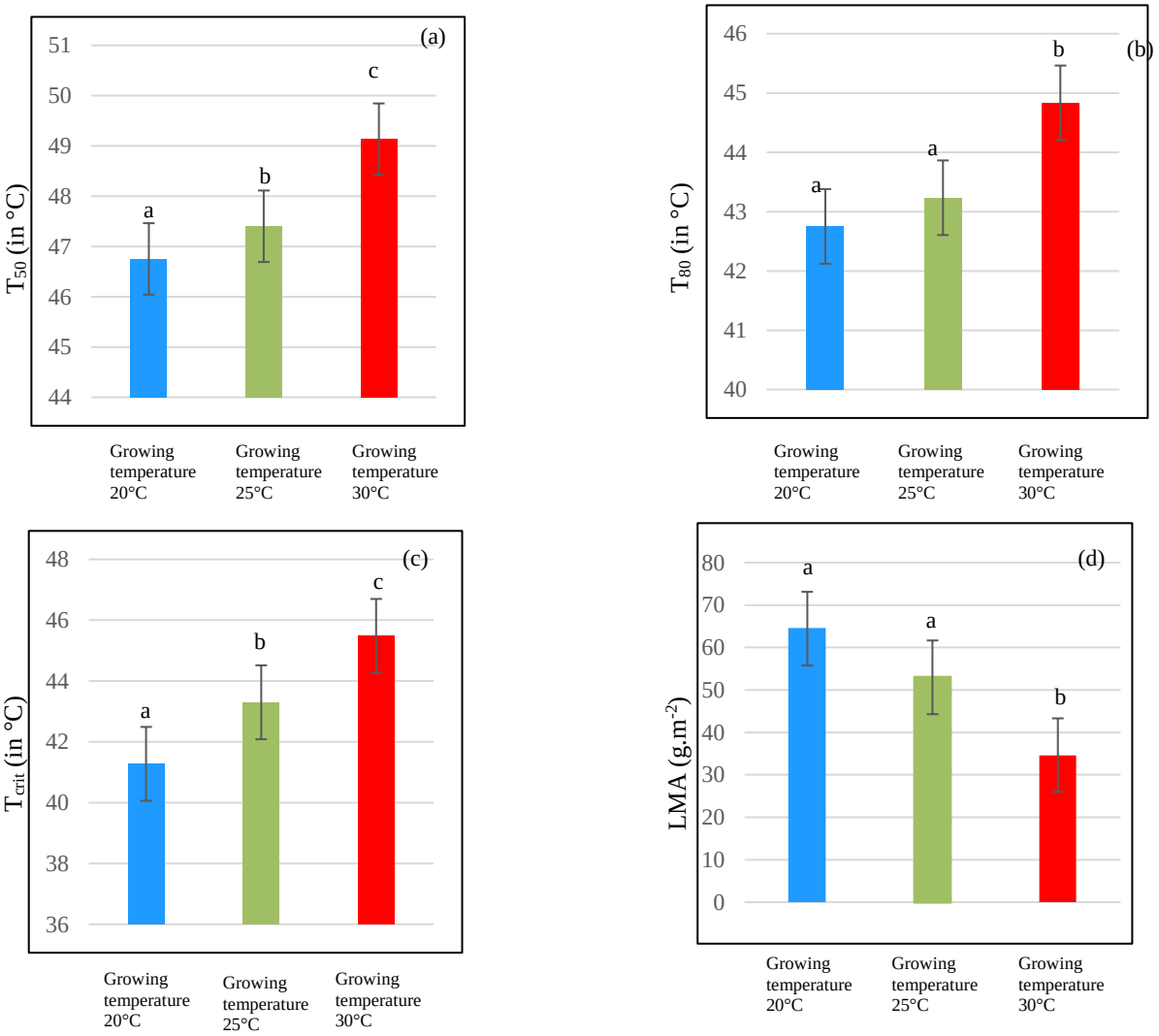


Figure 3: Mean values of $T_{50}$, $T_{80}$, $T_{crit}$ and LMA obtained for each leaf across the three temperature treatments. The blue bar plot here is the plants growing at 20 degree, the green one at 25 and the red bar plot correspond to 30°C. The small letters are the significant values observed (with a p value<0.05). The errors bars showed here are standard error: Mean (±SE). Panels (a) represent the average $T_{50}$ value across the three growing temperatures, (b) is $T_{80}$, (c) is the critical temperature's average and (d) is the LMA value.

*Comparison of Tcrit across the growing temperatures*

*Harungana montana* species showed different adaptations to heat tolerance according to which temperature they had grown. Figure 4 shows the rising of the minimal level of fluorescence, $F_0$ which was dependent on the temperature treatment. Beginning at the smallest temperature, for all growing temperatures, the value of $F_0$ was very small. Its value correlated with the temperatures rising. From 40°C, the temperature treatment at 30 degree started to be different from the two others by having a rise of the minimum level of fluorescence at a higher temperature.

By contrast, an unexpected result was the curves corresponding to treatment at 20 and 25 degree respectively which looks very similar to one another, but this is might be because the points showing here represent the average values obtained from the six plants. Another observation is that at 50 degree, for both 20 and 25°C, we observed a slightly decrease of $F_0$. We observed a significant difference of $T_{crit}$ among the three treatments ([F (2, 15) =34.2, p<0.001]). Following this, Tukey HSD was performed with a confidence level of 0.95. The results showed a  ficant difference in the critical temperature among the treatment at 20 and 30-degree Celsius ([F(2,15)=34.2, p<0.001]) between the one at 25 and 30 degree ([F(2,15)=34.2, p<0.01) as well as between 20 and 25 degree ([F(2.15)=34,2, p<0.01]).

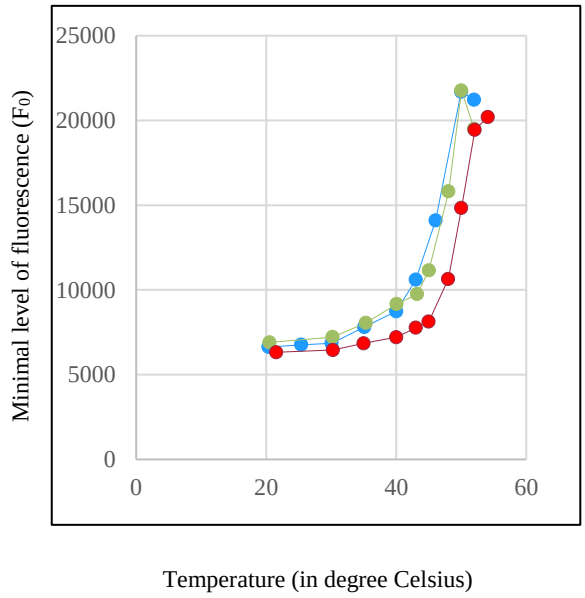


Figure 4: Comparison of the rising of the minimal level of fluorescence across the three growing temperatures. The blue circles are the plants which have grew at 20 degree, the green circles at 25 and the red circles correspond to a growth at 30 degree Celsius.

## *Temperature acclimation of leaf traits*

There was a significant negative correlation between the critical temperature and LMA ($p < 0.001$, Figure 5). We also performed an ANOVA test to see if the difference in $T_{crit}$ and LMA observed among the temperature treatments was significant. We observed a significant difference of LMA among the three treatments ([$F(2,15) = 15.68$, $p < 0.001$]). A Tukey HSD test showed a significant difference in LMA between the treatments at 20 and 30 degree Celsius ([$F(2,15) = 15.68$, $p<0.001$]) as well as between the one at 25 and 30 degree Celsius ([$F(2,15) = 15.68$, $p<0.05$]). However, between the treatments at 20 and 25 degree, no significant differences were observed. The difference observed for the critical temperature were significant for all the growing temperature as seen previously.

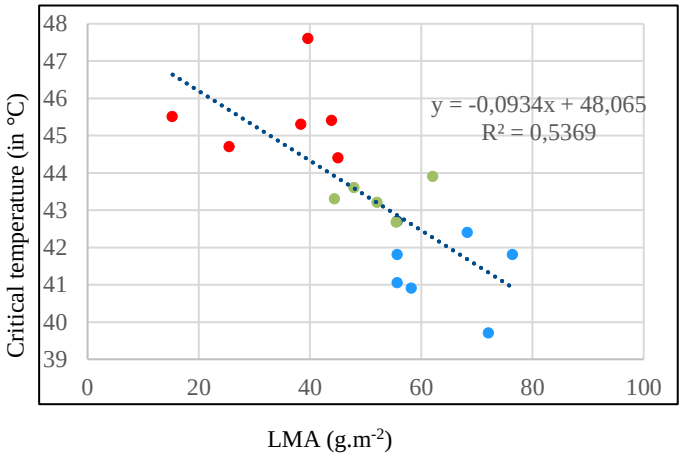


Figure 5: Critical temperature as a function of Leaf dry mass per area (LMA). Blue circles are the individuals acclimated to 20 degree, green at 25 and red circles correspond to plants growing at 30 degree Celsius.

## *Fatty acid composition analysis*

The plants contained different proportion of fatty acid class. In average, across the three growing temperatures they included 67% of MGDG, 25% of DGDG and 8% of PG.

As showed in figure 6, the average double bond index (DBI) was correlated with the critical temperature. Indeed, as the critical temperature increase (for the higher temperature treatment), the average double bond index decrease. Thanks to an ANOVA test, we observed that the differences in DBI for the three fatty acids class (MGDG, DGDG and PG) among the treatments were significant ([$F(2, 15) = 16.52$, $p<0.001$]); ([$F(2, 15) = 12.88$, $p<0.001$]) and ([$F(2, 15) = 4.85$, $p<0.05$]) respectively. The Tukey HSD test showed that the differences observed for MGDG and DGDG were significant between 20 and 30 degree ($p<0.001$) and between 25 and 30 degree ($p<0.05$), but not between 20 and 25 degree. By comparison, the differences observed for PG were only significant between 20 and 25 degree ([$F(2,15) = 4.85$, $p<0.05$]).

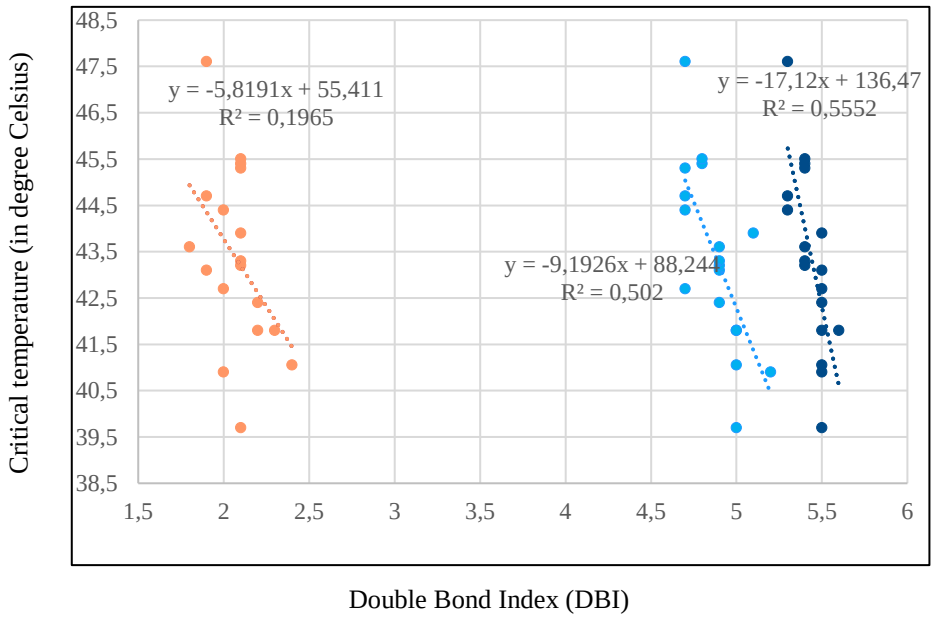


Figure 6: Negative correlation between the average double bonds of the fatty acids and the critical temperature. The dark blue line is the MGDG fatty acid. The blue one corresponds to DGDG, and the orange one is PG lipid class.

## V. Discussion

The main purpose of this study was to have a better understanding of the photosynthetic heat tolerance in the tropical tree species *Harungana montana*. This study found that the parameters measured ($T_{crit}$, $T_{50}$, $T_{80}$, LMA and fatty acid's degree of saturation) all acclimated to sustained growth temperature.

The first hypothesis anticipate that plants adapted to hot environments will show signs of greater quantum yield at high temperatures than plants acclimated to cooler temperatures. That means that species growing at the highest temperature should exhibit higher values for the $F_V/F_m$ ratio and therefore higher value of $T_{50}$, the temperature at which 50% of decline had been observed in the maximum quantum yield of photosystem II. This hypothesis was supported with $T_{50}$ being 0.24°C higher for a rise of 1°C of the growth temperature. This demonstrates that the acclimation is incomplete because it is not 1°C for each 1°C increasing in growth temperature. It indicates that plants will not be able to manage the future rise of temperatures and will therefore, be impacted by global warming. These results are in agreement with another study on the comparison of thermal tolerance on congeneric species between two different field sites. In that study, the authors showed that plant species had a high plasticity ability to photosynthetic thermal tolerance. This ability was explained as an adaptation to endure fluctuating habitats (Knight and Ackerly, 2002). In my study, the differences in $T_{50}$ were significant between all the temperature treatments (Figure 3). However, no significant differences in $T_{80}$ were observed between the treatment at 20 and 25°C (Figure 3). This is easily understandable as $T_{80}$ is the temperature at which 20% of decline is detected. This means that the performance of the plants is similar at moderately high temperatures. An explanation to this result

demonstrated in Figure 3, the plants acclimated to 20°C, have very large errors bars, showing a lot of variability.

The second hypothesis predicted that the critical temperature will be higher in individuals adapted to hot environments than plants growing under lower temperatures. This hypothesis was supported with $T_{crit}$ being approximately 0.4°C higher for a rise of 1°C of the growth temperature (Figure 3). This conclusion is in agreement with previous studies done on seasonal variations of the critical temperature on plants where the highest $T_{crit}$ values were exhibit by plants coming from tropical or semiarid regions compared to others coming from temperate climate which displayed the lower $T_{crit}$ value (Zhu et al., 2017). However, in the study by Zhu et al., species from tropical forests displayed a lower degree of acclimation than ours. Indeed, their critical temperatures raised between 0.20-0.22 °C for each 1°C gaining in growth temperature. However, despite this acclimation dependent on temperature, from a certain upper limit of the photosynthetic heat tolerance of PSII, extreme heat will be fatal to the plant species even for tropical trees adapted to very high temperatures. Additional research is required in order to determine if there are other parameters than the thermal habitat which can command this upper limit (Zhu et al., 2017). Another relevant point is the decline of $F_0$ around 50 degree Celsius, for the treatment at 20 and 25°C. As it has been explained in a scientific paper dealing with the high temperature dependence of $F_0$, at 50°C, the minimum level of fluorescence reaches $F_m$, the maximum value of fluorescence. Therefore, from this temperature $F_0$ will start to follow the same template as $F_m$: meaning decrease due to heat damage (Pospìŝil et al., 1997).

The third assumption predicted that plants growing at high temperatures would have lower leaf dry mass per area (LMA), allowing them to have a better heat conductivity and therefore, a better heat avoidance. This assumption was supported in our study with plants growing at the lowest temperature, having LMA value of 29.80 $g.m^{-2}$ higher than the ones living at 30-degree Celsius (Figure 5). In addition, a low leaf mass per area will lead to a small density allowing the leaves to display a larger size and therefore heat will evacuate more effectively thanks to the morphology of the leaf.

These results were not supported with previous finding by Sastry and Barua, 2017. Their study on the leaf thermotolerance in tropical trees showed that species with high LMA had higher thermal tolerance. However, in that study, research was done on among species variation while I was only working with one species. Therefore, it is difficult to make a comparison between the two studies' results. By comparison, my results were supported by another research work (Poorter et al., 2009). In this review, a meta-analysis on LMA was done. The results showed that at low temperature, there was less space for the cells in the leaf tissue, therefore, there was a large number of small cells per unit area. That's also mean more cells walls per unit area and so the leaf volume and density will increase with the leaf dry mass per area (Poorter et al., 2009). Likewise, the relationship between LMA and thermal tolerance may exist because of a low LMA value may be linked with a higher photosynthetic activity and therefore, higher stomatal conductance (more water in the form of vapor exits through the stomata of the leaf) and transpiration. This will allow to lower the leaf temperature and therefore, improve the heat tolerance. However, these did not vary among the plants in my study because they were in growth chambers. So, for this study the LMA change seems to be only due to growth temperature. Despite the significant differences in LMA found between 30 and 25°C as well as between 20 and 30°C, no significant differences were observed between 20 and 25°C in this study. This observation could be explained by a high level of variability in LMA for the plants growing at 20°C (Figure 3). This variation can be explained by the fact that the leaf size and density were very different across one plant, so during the measurements leaves of random size were selected.

The fourth and last hypothesis forecast that the heat tolerance was correlated with a variation of leaf's lipids physical properties. Therefore, plants acclimated to the highest temperature treatment, had a

greater saturation level of fatty acids to maintain a certain stability in case of extreme heat (seen as a reduction in the double bond index), than plants growing under cooler temperatures. As a result of that, the cell membranes will be more fluid (Zhu et al., 2017). This assumption was supported by our study, where we found that the average double bonds number of MGDG, DGDG and PG was 0.21 lower for 10°C of temperature difference (between 30 and 20°C, Figure 6).

This result was similar to another study in which a greater thermal tolerance in a mutant of *Arabidopsis thaliana* exhibited a great quantity of palmitic acid, the most saturated fatty acid. In that study, above 28°C, the mutant grew quicker than the wild type, showing that its different fatty acid composition will lead to a better tolerance toward extreme heat (Kunst et al., 1989). A similar study done on regulation of membrane fatty acid composition by temperature in a mutant of Arabidopsis showed that particular quantities of saturated and unsaturated fatty acids are needed to tolerate heat (Falcone et al., 2004). A study done on plant adaptation to different temperatures showed that plants change the degree of saturation of lipids membrane to confront with occasional temperatures variations. However, they only used their lipid remodelling to confront repeated temperature variations. For instance, lipid adjustment thanks to the transfer of head groups which is very fast and cost a low amount of energy (Zheng et al., 2011).
Future studies should be done in order to determine if other factors, in the leaf tissue than fatty acids and LMA could influence the variation of critical temperature and thus, impact heat tolerance. Already, the study done on plasticity of photosynthetic heat tolerance in plants demonstrated that 40% of variations observed of $T_{crit}$ can be due to lipids composition among the leaf (Zhu et al., 2017). These results are pretty similar to my finding with the correlation of $T_{crit}$ and DBI where $R^2$ was more than 0.5 for two majors' lipids group (MGDG and DGDG).

As showed above, to improve the plant performance and survival at high temperatures, they adapted by changing their membrane lipid composition and LMA. Even by increasing tolerance or by reducing the heat in order to decrease the need in high tolerance (avoidance). Thus, these results showed that this tropical tree species does both. This is interesting because this avoidance could explain why $T_{crit}$ and $T_{50}$ increase so much less than air temperature. The increase in $T_{crit}$ and $T_{50}$ should be more closely related to the increase in leaf temperature. So, if the leaf temperatures increase more slowly than air temperature, then $T_{crit}$ and $T_{50}$ don't have to keep up with the air temperature increase to survive. Therefore, a future measurement that can be performed to test the effect of LMA on high temperature avoidance is what the leaf temperatures would have been in plants from different growth temperatures if they were exposed to same amount of heating. Indeed, we expected to have a lower leaf temperature for the plants coming from the higher temperature treatment as it will have a lower LMA and therefore, a high temperature avoidance.

## VI. Conclusion

The results observed here showed that the determination of the critical temperature and the ratio $F_V/F_m$ allow us to support our statement about the ability of plants growing under different temperatures, to acclimate their photosynthetic heat tolerance. In fact, our results show plants grown at higher temperatures are better able to tolerate heat with a higher $T_{50}$ and $T_{crit}$, corresponding to individuals adapted to hot environments. However, that acclimation was less than 1:1 ratio meaning plants suffer as temperature will progress to increase. The second part of our study had demonstrated that the different heat tolerances observed among the 3 treatments is explained by a variation in fatty acid's saturation level and leaves mass per area. Our results exhibited a higher level of saturation of lipids and lower LMA in plants acclimated to warmer habitats.

This study provided a solid confirmation concerning the capability of plants which have grew under different temperatures, to react contrastingly in case of exposition to extreme temperatures. Thanks to this adaptation, species acclimated to higher temperatures might have better chance of survival in a global warming perspective compared to other plants. Although, this statement is true provided that the temperature stays below a certain threshold in order to not damage the plant. In fact, above this limit, extreme heat waves might cause irreversible negative effects on plant efficiency. Indeed, as it has been showed in our study, that even the plants adapted to the highest growing temperature undergo serious damages under extreme heats. Therefore, it can be concluded that the rising of temperatures due to the global warming will cause negatives effect on most of the plant's species and in consequence on the whole ecosystem.

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