

# Variation in thermotolerance in woody plants from a seasonally dry tropical forest

Thesis submitted towards the partial fulfilment of BS-MS Dual degree  
programme

By

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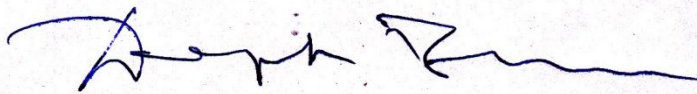
(IISER Pune)



Indian Institute of Science Education and research, Pune

## Certificate

This is to certify that this dissertation entitled "Variation in thermotolerance of plants in a seasonally dry tropical forest" towards the partial fulfilment of the BS-MS dual degree programme at the Indian Institute of Science Education and Research, Pune represents study/work carried out by Kavya Mohan.N at Indian Institute of Science Education and Research(IISER), Pune under the supervision of Dr. Deepak Barua, Assistant Professor, Biology division, IISER Pune during the academic year 2016-2017



Signature of the Supervisor

Dr. Deepak Barua

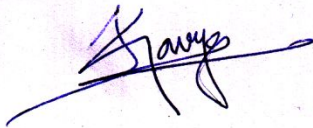
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## **Declaration**

I hereby declare that the matter embodied in the report entitled "Variation in thermotolerance of plants in a seasonally dry tropical forest" are the results of the work carried out by me at the Department of Biology, Indian Institute of Science Education and Research(IISER), Pune, under the supervision of Dr. Deepak Barua and the same has not been submitted elsewhere for any other degree.



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## Abstract

Temperature is expected to increase by 3 to 6°C by 2100 in tropics. Tropical species which are already near their upper temperature threshold are expected to experience temperature above their tolerance limit. Tropical forests are major carbon sink and their decline can have a positive feedback on global warming. So it is important to study the effect of higher temperatures on tropical tree species so as to predict forest dynamics under future high temperature. This study tries to understand the variation in thermotolerance of 38 coexisting woody perennial species that differ in leaf habit (evergreen and deciduous) and life form (shrubs, lianas and trees). I also check for ability to recover from heat damage and the possible correlation between thermotolerance and key leaf functional traits. The temperature at which performance drops to 50% ( $T_{50}$ ) ranged from 44.5 to 50.5°C. Lianas and trees showed higher thermotolerance than shrubs. The evergreen tree species had higher thermotolerance than deciduous tree species. More over a positive correlation was found between thermotolerance and LMA and LDMC. Most species did not show any recovery from heat damage. The maximum reported temperature of the region is close to the  $T_{50}$  of many species and they are at the risk of irreversible leaf damage with predicted increase in temperature. Species with high LMA and LDMC are expected to get an advantage over other species in the future warmer climate.

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## **Acknowledgement**

I would like to thank Dr. Deepak Barua for guidance and support. I would like to thank Shubham Chhajed, Gauri Binayak and Ron Sunny for their help in the experiment. I would like to thank Kalu and Ganapat for their help in sample collection. I would like to thank Aniruddh Sastry, all other lab members and Asmi Jezeera for their support and suggestions.

## Introduction

Temperature plays an important role in growth and functioning of an organism. Besides that, features like geographic distribution of species is often restricted by the temperature extreme or the temperature outside its tolerance limit (Araújo *et al.* 2013). Plants being sessile cannot escape the changes in their environment and needs to adapt or find strategies for survival. Temperature is expected to increase by 3 to 6°C in tropics by 2100. Species might see temperatures above their tolerance limit in their present habitats. Tropical species, which are already near their tolerance limit, are expected to get affected the most (Tewksbury *et al.* 2008). So it is important to study thermotolerance of species, especially from tropics. There are relatively fewer studies that examine thermotolerance of plants, especially tropical plants.

Species show maximum performance under optimum temperatures. But high temperature extremes results in reduced performance and growth, cellular/tissue damage and ultimately mortality (Teskey *et al.* 2015). Thermotolerance is defined as tolerance towards temperature extremes. Thermotolerant species are able to perform better in high temperature environments compared to less thermotolerant ones. So thermotolerant species can expand their geographic range to high temperature regions. The field of plant thermotolerance is very old dating back to early 20<sup>th</sup> century. Different methods like response of photosynthetic gas exchange (Berry & Bjorkman, 1980), electrolyte leakage (Anderson *et al.* 1990), visual assessment of leaf damage (Buchner *et al.* 2013) and chlorophyll fluorescence (O'Sullivan *et al.* 2017; Curtis *et al.* 2016) are used for studying thermotolerance.

Temperature is expected to increase by 3 to 6°C in tropics by 2100 ( Malhi *et al.* 2014). The duration and frequency of extreme temperature and drought events are also expected to increase. With the predicted increase in temperature, many species might see temperatures above their tolerance limits in their present geographic ranges. Range shifts of plants may not be able to keep up with the present rate of increase in temperature (Nathan *et al.* 2011). Temperature increase can give an advantage to high thermotolerant species over the low thermotolerant species in the same geographic range.

Tropical species are shown to have comparatively high thermotolerance (O'Sullivan *et al.* 2017) , but tropical species are already near their upper tolerance limit



(Doughty & Goulden, 2009). Even though there is an increase of 20°C in maximum temperature from the pole to the equator, the increase in critical temperature of PSII function is only 8°C (O'Sullivan *et al.* 2017). Moreover, tropical species have seen relatively fewer temperature fluctuations in their evolutionary history and are adapted to a narrow range of conditions (Janzen 1967). So, they are expected to have less potential for adaptation. This makes tropical plant species highly vulnerable.

Studies which compare tropical and temperate species have shown that tropical species have higher thermotolerance (Cunningham & Read, 2006). O'Sullivan *et al.* (2017) showed an increase in thermotolerance from arctic region to tropics. This study showed a huge variation within site, with some species from tropics having lower thermotolerance than that in the arctic. Although species from warmer habitats are shown to have higher thermotolerance, ecotypes vary in their thermotolerance (Knight & Ackerly, 2002; Jen Hsien Weng *et al.* 2006). About 20°C difference in critical temperature for the function of photosystem II was seen within latitude in the O'Sullivan *et al.* (2017). Differences in habitat or microclimate can be one of the reasons for this (Curtis *et al.* 2016) But there are only a few studies which look at a large number of species from the same microclimate. Within microclimate itself, the individual, recent environment conditions like season or other stress can cause a change in thermotolerance. We don't understand the reasons behind all these variations in thermotolerance properly. We don't understand how these will effect response to global warming.

The difference in strategies of plants in coping up with thermal stress can be the reason behind these variations. For example, species which are dormant or in seed phase during the hottest period does not need high thermotolerance. Variation in leaf habits ( evergreen or deciduous) and leaf functional traits such as leaf mass per area (LMA), leaf dry matter content (LDMC), thickness and area can be possible explanations for the within site variations. The plant functional types can also be a possible reason for this variation.

Leaf functional traits such as leaf mass per area (LMA) and leaf dry matter content (LDMC) represent the carbon investment in leaf. LMA is shown to be related to the

ecological strategy of plant species (Wright *et al.* 2004). Understanding relation of thermotolerance to different leaf traits will help us to make general predictions about survival of species during the future climate change. There are contrasting results on the correlation of thermotolerance to LMA. Knight & Ackerly (2003), Gallagher (2014) and Sastry unpublished (2017) shows a positive correlation between LMA and thermotolerance while Godoy *et al.* (2011) shows a negative correlation and Zhang *et al.* 2012 showed no correlation. Leigh *et al.* (2012) and Groom *et al.* 2004 shows that leaf thickness shows a protective role from heat damage. High thickness and small area are considered thermal protective traits (Curtis *et al.* 2012). Leaf habit (evergreen and deciduous leaf phenology) is expected to be related to thermotolerance - species which have leaves all throughout the year sees all the temperatures and are expected to be more thermotolerant than deciduous species which remain leafless for most of the summer. Sastry (unpublished, 2017) shows evergreen species to be more thermotolerant than deciduous species. O'Sullivan *et al.* (2017) however reports that the grouping into evergreen and deciduous did not show any effect.

Plants are classified into different life forms mainly based on the life span and morphometric feature of species (Pirson & Zimmermann, 1982). Different life forms coexist within a community. They vary in many functional properties. Annual herbs are less thermotolerant compared to woody perennials (Downton *et al.* 1984). Trees, lianas and shrubs are major constituents of tropical forests. They vary in leaf traits and water uptake which can have an effect on their actual temperature and thus thermotolerance. There is not enough knowledge about thermotolerance of lianas and variation in thermotolerance between life forms. Doughty (2011) studied the effect of branch warming in trees, gap species and lianas and showed a decrease in maximum photosynthesis in trees.

$F_v/F_m$  is one of the widely used measure for estimating thermotolerance. It gives the maximum quantum yield of photosystem II function and PSII is considered to be the most heat sensitive part. The light absorbed by PSII can be used for photochemistry, fluorescence or heat loss and these are in competition with each other. The dark adaptation ensures that the primary quinone acceptor( $Q_A$ ) of photosystem II reaches its maximum oxidised state and PSII is capable of photochemical reduction of  $Q_A$  and thus minimum fluorescence in presence of a weak measuring light. Rapid exposure

of high intensity saturation pulse leads to the maximal reduction of  $Q_A$  leading to maximum fluorescence.  $F_v/F_m$  is the ratio of variable fluorescence ( difference between maximum and minimum fluorescence) to maximum fluorescence. It is an indication of the capacity of PSII to accept light.

This study tries to understand the variation in thermotolerance in coexisting species within a habitat in tropics. I try to understand underlying factors that drive the variation between species. I ask a) Whether coexisting species vary in thermotolerance b) Does life form of the species have an effect on variation c) Do species show a recovery from heat damage d) Do individuals from open and edge habitats differ in thermotolerance e) Do leaf habit and leaf traits have an effect on variation.

## **Materials and methods**

### **Study area**

This study was conducted in Bhimasankar wildlife sanctuary located in Pune district, Maharashtra, India (19.1320° N, 73.5540° E, altitude 900m) and part of Northern Western Ghats. It is a protected area spanning 131km<sup>2</sup> of evergreen to dry deciduous forest which is highly fragmented.

The important seasons are hot dry summer (March to May) and monsoon (June to September). Bhimasankar receives an annual rainfall of about 3000mm, concentrated in the months of June to September and months April and May mark the driest time of the year. The mean monthly temperature ranges from 7°C in December to 36°C in May. Absolute maximum temperature recorded in the region between 2014 and 2015 is 42.1°C.

The collection site was in Nigdale on the eastern slope of Bhimasankar and has 3 habitats-open, edge and closed. Open forests or crest forests are characterised by short trees, less cover and low soil moisture content. Closed forests or valley forests have tall trees, high tree cover, high soil moisture content. The edge forest is an intermediate of these two. A representative image of habitat structure of Bhimasankar is given in the appendix. This study was done in open and edge habitats. All woody perennial species in these two habitats with leaves during the monsoons were collected for the study.

## **Sample collection**

Leaves were collected from six mature individuals of all available woody perennial species in open and edge habitats from 14<sup>th</sup> August 2016 to 16<sup>th</sup> September 2016. The species collected included species belonging 3 life forms –trees, shrubs and lianas. Three representative annual herbs were also collected. Five species were collected from both open and edge to check if these habitats have an effect on thermotolerance. Individuals were randomly selected from mature healthy individuals. Species above girth at breast height (GBH) cut offs for each life forms were considered mature. For trees the cut off was 30cm for lianas and shrubs it ranged from 5cm to 10cm depending species. The species cut offs were available from previous studies in the lab. The names of the species which were collected are given in the appendix.

First fully mature sun leaves were collected from canopy level. When the canopy leaves were not accessible, leaves from side branches were used. Leaves from gaps were collected for under canopy shrubs. 12 to 15 individuals were collected in a day and 15 leaves were collected from each individual for temperature response assay and leaf traits combined. Collected leaves were kept in wet paper bags in ziploc bags and were taken to the lab. Leaves were cleaned and were kept for overnight saturation using petiole dip method and were kept inside a Ziploc bag containing water for maintaining humidity.

## **Temperature tolerance assays**

Temperature tolerance assays were done on the day after collection day. Discs of 0.8cm radius were punched using cork borer. Discs covered in a muslin cloth and aluminium foil were sealed inside Ziploc covers with moist tissue paper and these were heated in a temperature controlled refrigerated JULABO F25 MC water bath. Discs were heated at 7 different temperatures including control. The temperatures used were 25, 40, 42.5, 45, 47.5, 50 and 52.5 °C. A dummy leaf disc was used to monitor temperature during treatment. Discs were sandwiched in between black cloth covered with thick paper for dark adaptation for 30 minutes immediately after heat treatment.  $F_v/F_m$  measurements were taken using PAM2500 fluorometer.

The discs were sealed in a Ziploc bag with water on moist tissue in a plate. The discs were placed on moist tissue paper in a plate, inside a Ziploc containing water to maintain humidity.  $F_v/F_m$  measurements were repeated after 24 hours.

Chlorophyll a fluorescence measurements were taken using pulse amplitude modulated fluorometer (PAM 2500 fluorometer). Maximum chlorophyll fluorescence ( $F_m$ ), minimum fluorescence ( $F_0$ ), and the ratio of variable to maximum fluorescence ( $F_v/F_m$ ) were used for quantifying thermotolerance. Variable fluorescence ( $F_v$ ) is the difference between maximum and minimum fluorescence.  $F_v/F_m$  gives the maximum quantum yield of PSII photochemistry and is an indicator of the integrity of photosynthetic apparatus. A decline in  $F_v/F_m$  means damage in photosynthetic apparatus. The temperature at which a 50 % reduction in  $F_v/F_m$  occurs is used as an indicator of heat tolerance.

### **Leaf traits**

Leaf thickness, leaf area, leaf mass per area (LMA) and leaf dry matter content (LDMC) were measured. Fresh weight of 6 water saturated leaves was measured on the same day of thermotolerance assay. Leaves were scanned using CanoScan Lide 110 for area calculation and were later calculated using ImageJ. 0.8cm radius discs were taken from each leaf and they were weighed separately. Dry weight for both leaves and discs were measured after drying for 4 days in hot air oven at 60°C. Leaf mass per area (LMA) was calculated as the ratio of dry weight and area. Leaf dry matter content (LDMC) was calculated as the ratio of dry weight to fresh weight. LMA and LDMC were calculated separately for both disc and whole leaf. Leaf thickness was measured using screw gauge.

### **Statistical analysis**

Temperature response curve was generated for day0 and day1 measurements of each individual and at species level by fitting the  $F_v/F_m$  values to 4 parameter logistic sigmoidal curve with lower asymptote set to zero using R package drc (Ritz and Streibig 2005). The temperature at which  $F_v/F_m$  drops to half of control value ( $T_{50}$ ) was estimated from best fit curve.  $T_{50}$  was used as a measure of heat tolerance.

The  $T_{50}$ s and all leaf trait values were checked for normality using Kolmogorov Smirnov test.  $T_{50}$  for day0 and day1 were normally distributed. All the leaf traits

deviated from normality. For leaf thickness and area, ln transformation was used. For LMA leaf, LMA disc, LDMC leaf and LDMC disc box cox transformations with  $\lambda$  0.2306, 0.2217, 1.0311, 1.3887 were used respectively. Kolmogorov Smirnov test was done again on the transformed data and all were normally distributed.

ANOVA with species nested within life forms was used for evaluating the effect of life forms on thermotolerance. A repeated measures ANOVA was used to check if thermotolerance measurements as  $T_{50}$  measured on day1 differed from that of day0. Species collected from both open and edge were used to check if the habitats matter. ANOVA was used to check if habitat from which species was collected has any effect on thermotolerance. ANOVA with species nested within leaf habit (evergreen and deciduous) was used to evaluate the effect of leaf habit on thermotolerance. Pearson correlation was used to assess the relation between thermotolerance and leaf traits (leaf thickness, area, LMA, LDMC). All statistical tests were done using STATISTICA (Version 9.1, Statsoft)

## Results

Maximum quantum yield of PSII ( $F_v/F_m$ ) decreased to zero or close to zero within the range of 40°C to 52.5°C for all the 38 species studied. There was no significant decrease between 25°C and 40°C. Only a few species maintained a nonzero  $F_v/F_m$  at 52.5°C, most species dropped to zero at 50, and a few sensitive ones reached zero at 47.5°C.  $T_{50}$  (the temperature at which maximum chlorophyll fluorescence becomes half of the control temperature) ranges from 44.5°C to 50.5°C. *Lasiosiphon eriocephalus* (LE) showed the lowest  $T_{50}$  and *Olea dioica* (OD) showed the highest  $T_{50}$ . Representative temperature response curves of species belonging to different life forms are shown in figure1. Figure2 shows the variation between species in each life form. Thermotolerance of shrubs ranged from 44.5 to 49.2°C lianas 45.6 to 49.8°C, and trees 44.6 to 50.4°C (figure2).

The woody perennial species had significantly high thermotolerance compared to annual herbs. The mean of thermotolerance of all woody perennials was 47.8°C and that of herbs was 44.03°C. Among woody perennials, shrubs had a lower thermotolerance compared to lianas and trees. Lianas and trees did not significantly differ in their thermotolerance (figure3). Both  $T_{50}$  measured immediately after heat



treatment ( $T_{50}$ ) and that measured 24 hours after heat treatment ( $T_{50}$  Rec) showed similar results.

Species varied in their ability to recover from damage caused by heat treatment (figure 4, table 2). There was no recovery of  $F_v/F_m$  for most of the species. But about 5% of the species showed recovery and for another 5%, the  $F_v/F_m$  value decreased further. The species which showed recovery showed only less than 1°C rise in  $T_{50}$ . So irreversible heat damage is happening even at 42.5°C. Both  $T_{50}$  measured immediately after heat treatment and that measured 24 hours after heat treatment ( $T_{50}$  Rec) showed similar results in all comparisons.

Thermotolerance of individuals of five species which were collected from both edge and open habitats did not differ significantly (figure 5, table 3) meaning the difference in the microclimate between these habitats is not strong enough to have an effect on thermotolerance. So we can consider species collected from both habitats as from the same microclimate.

Evergreen tree species had a higher thermotolerance compared to deciduous tree species (figure 6, table 4). Even though significant, the difference between the sets is very low. There were 10 evergreen and 10 deciduous species and the most thermotolerant one in the entire list is also an evergreen tree.

Thermotolerance showed a positive correlation with LMA (disc and leaf) (figure 7) and LDMC (disc and leaf) (figure 8). We could not find a correlation for leaf thermotolerance with leaf area and thickness. Spearman correlation coefficients are given in table 5 and Pearson correlation coefficient is given in appendix table 2.

## **Discussion**

Thermotolerance measured as  $T_{50}$  ranged from 44.5°C to 50.5°C in 38 species which were studied. The  $T_{50}$ s of least thermotolerance species are just 2.5°C above the reported highest air temperature of 42.1°C of this site. Drought and summer coincide, so the option of transpirational cooling may not be available. Given that the leaf temperature can go higher than the air temperature in absence of transpiration (Vogel, 2005) some of these species might be already seeing these high temperatures. With the predicted increase in temperature half of these species will

see air temperatures above their  $T_{50}$ s which mean their leaves might not be able to survive hot dry summer.

Trees and lianas had a higher temperature tolerance compared to shrubs. This was unexpected. Lianas which have longer roots show higher water uptake and generally don't suffer from drought and they have high transpiration rate (Schnitzer 2005). So they are expected to be at lower leaf temperature compared to trees and shrubs. And therefore less probable to get selected for higher thermotolerance. Even though significant, the difference between the mean of the lifeforms is low. This can be because of the huge variation within life forms. The woody perennial life forms had compared to the representative herbs as expected (Bilger *et al.* 1984). The range of  $T_{50}$  in tree species was similar to Sastry (unpublished 2017) work in Western Ghats which reported a range of thermotolerance of 45 to 51°C. Curtis *et al.* 2016 reported a higher  $T_{50}$  range of 47.9 to 53.2 for woody species in an arid region.

For most of the species there was no recovery from heat damage after 24 hours from heat treatment confirming that the treatment caused irreversible damage. The heat damage caused even by 42.5°C (the first temperature where a decline in  $F_v/F_m$  happens) is a permanent irreversible damage. The heat treatment that we give here is for 30 minutes. This relatively less destructive temperature can cause more damage if it is seen for a longer time (Sutcliffe 1977). This site could see temperature above 42.5°C in near future. This means many species in the study site will experience a widespread loss of leaves to heat damage in the near future.

There is no significant difference between edge and open habitats which actually lies next to each other in space. So, we can consider species from both these habitats as the from same microclimate and are competing for same resources. There is a 6°C difference in the  $T_{50}$  of species which sees the same environmental characters. Some species will be more negatively affected compared to others in the increased temperature condition. It is possible that during drought when soil moisture content of these two habitats differs, the species from these two habitats may respond differently.

Evergreen trees had higher thermotolerance compared to deciduous trees similar to Sastry (unpublished 2017). This differs from O'Sullivan *et al.* (2017) which shows no relation between leaf habit and thermotolerance which included data from different

biomes. The species examined here are broad leaved evergreen and drought deciduous species. It can be one of the possible reason for this difference. In the high temperature condition this broad leaved evergreen species might get an advantage over coexisting drought deciduous species. Although a significant relation was seen between life form and thermotolerance, the difference between evergreen and deciduous was small probably because of huge variation within these leaf habits.

LMA showed a positive correlation with thermotolerance similar to Sastry (unpublished 2017) from Western Ghats. This differs from O'Sullivan *et al.* (2017) which showed no correlation between LMA and thermotolerance for species from different biomes. This may be because this correlation is absent in lower temperature regimes. But this result also differs from Zhang *et al.* (2012) conducted in a tropical site and showed no correlation. LDMC also showed a positive correlation with thermotolerance. Species with high LMA and LDMC represent the slow growing conservative strategy and low LMA are generally fast growing acquisitive species. This suggests that the future forests will have more evergreen, high LMA species and the low LMA deciduous species might get outcompeted. The tropical forests are a major carbon sink. If the species of tropics prefer a less productive, slow growing strategy, it might lead to a positive feedback to rise in CO<sub>2</sub> concentration and thus global warming.

## **Conclusion**

There is variation in leaf thermotolerance between species in the same microclimate. Many species are in immediate threat of irreversible leaf damage. Some of this variation maybe explained by leaf habit and leaf functional traits. Species which are evergreen and has high LMA and LDMC may get an advantage over the others in future climate change condition.

## Figures and tables

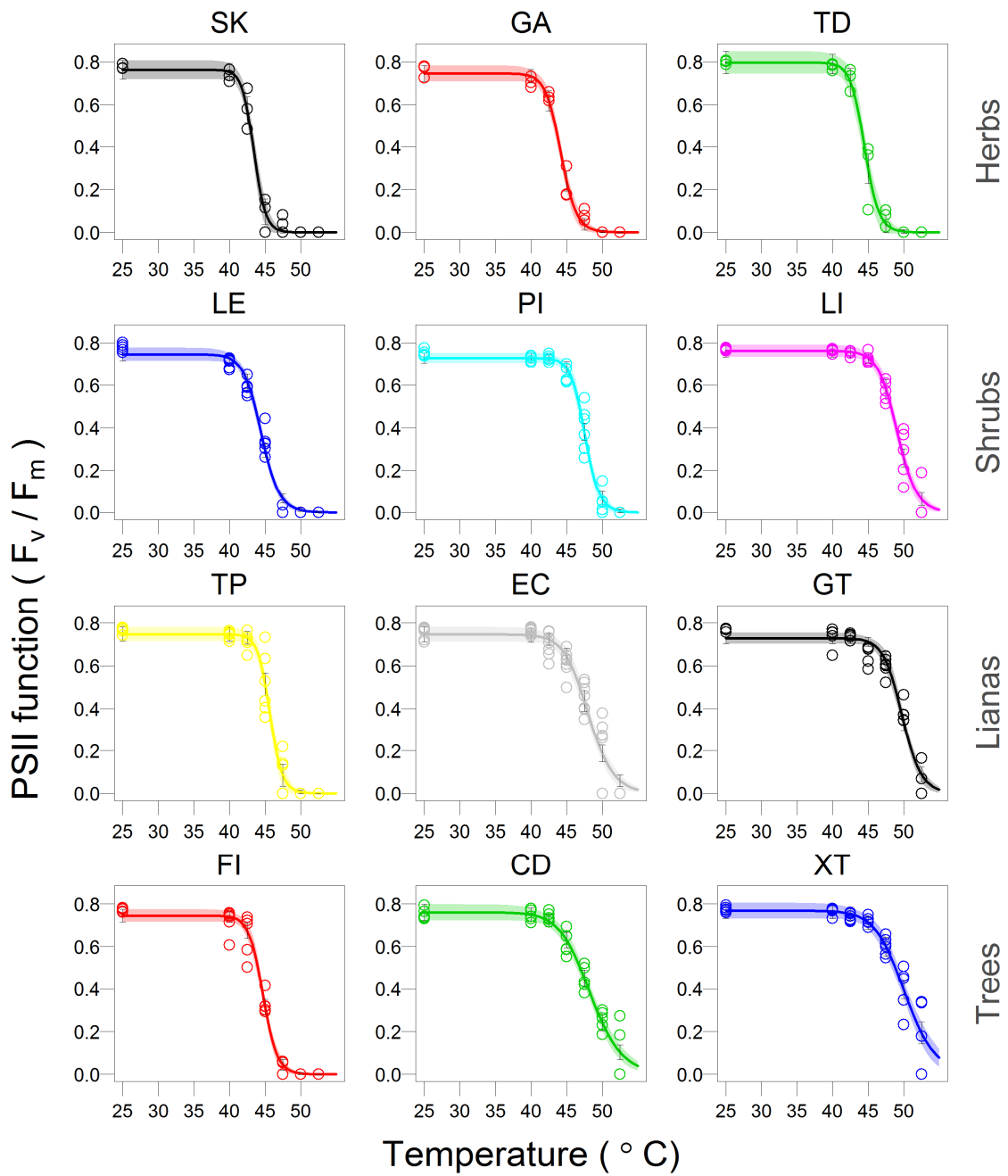


Figure 1: Representative temperature response curve of a) herbs, b) shrubs, c) lianas and d) trees. X axis is temperature and Y axis is  $F_v / F_m$  (maximum quantum yield of PSII photochemistry). Error bars represent 95% confidence interval.

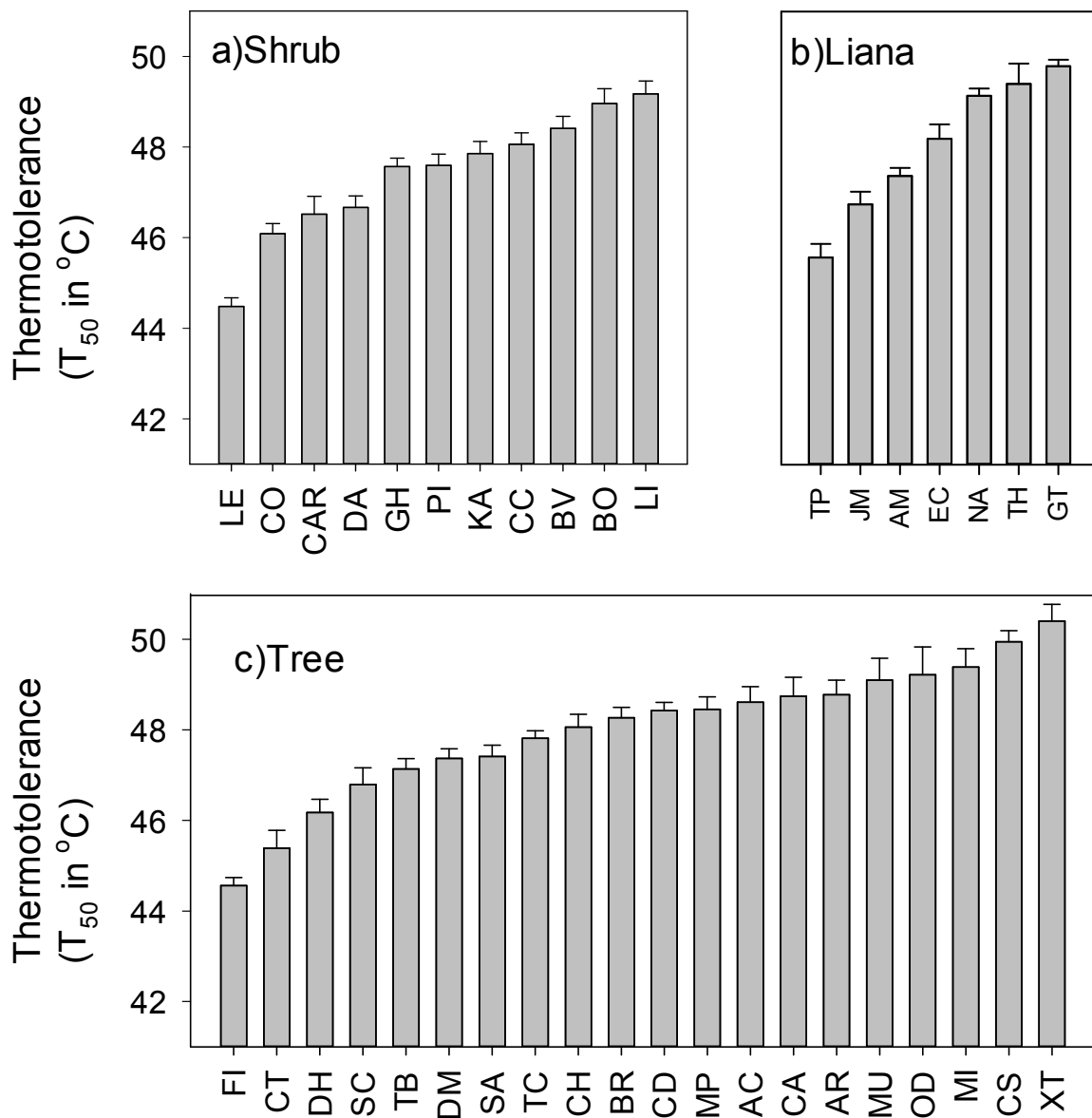


Figure 2: Variation in thermotolerance in species belonging to different life forms 2a) Shrub b) Liana c) Tree. Each bar represents mean for respective species. Six individuals were used for all species except CO(9), LI(5), EC(7). Error bars represent standard error of mean.

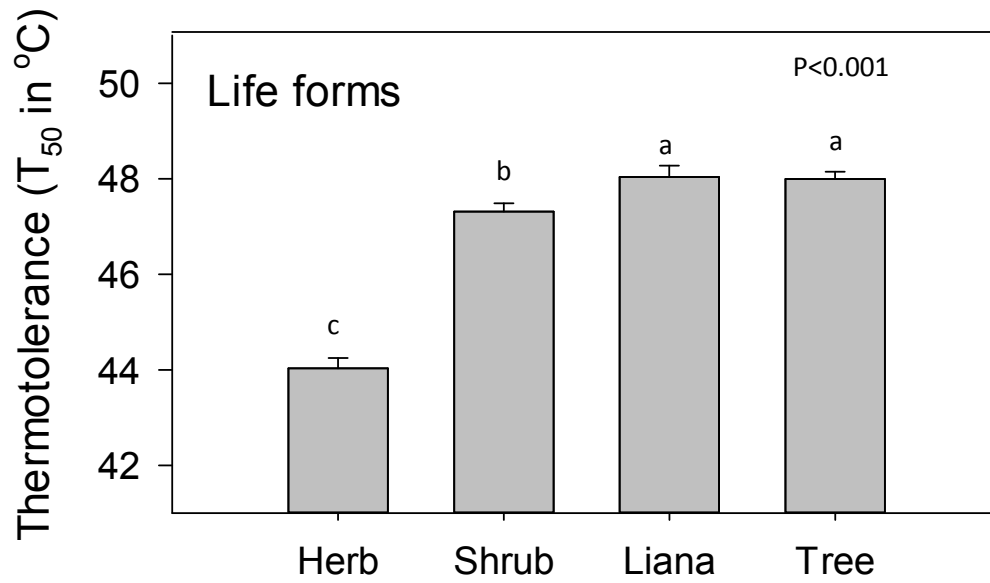


Figure 3: Variation in thermotolerance( $T_{50}$ ) across life forms. Lianas and trees are more thermotolerant than shrubs. Bars represent mean of all shrubs ( $n=11$ ), lianas ( $n=7$ ) and trees( $n=20$ ) in the habitat and three representative herbs.



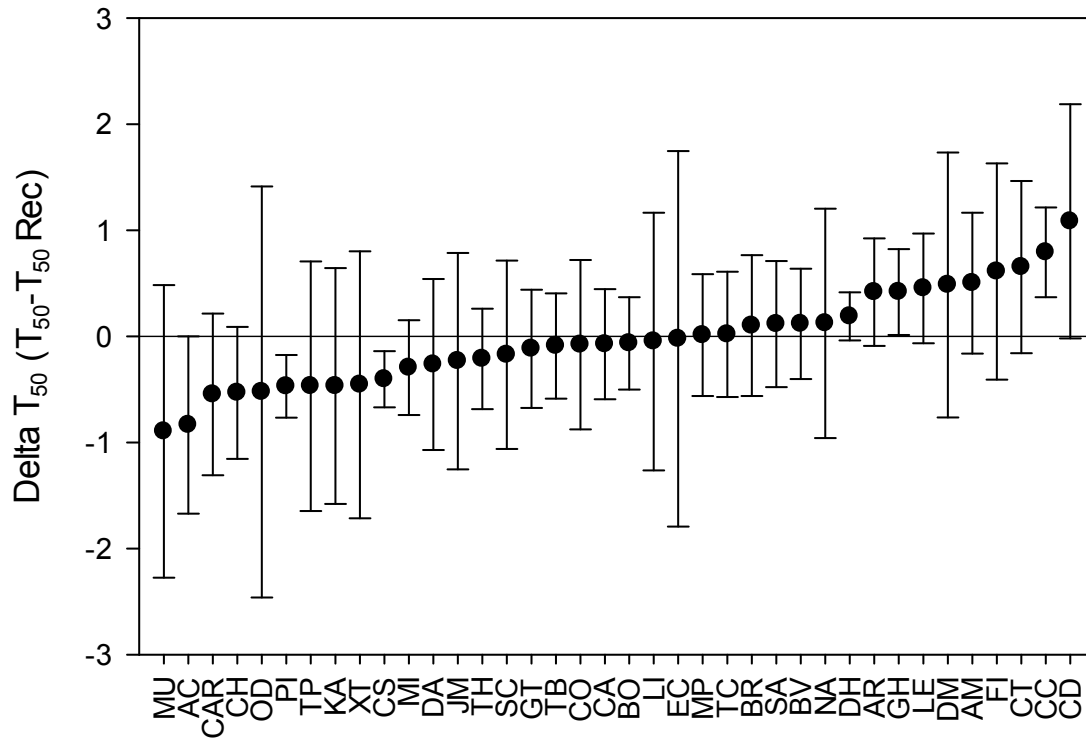
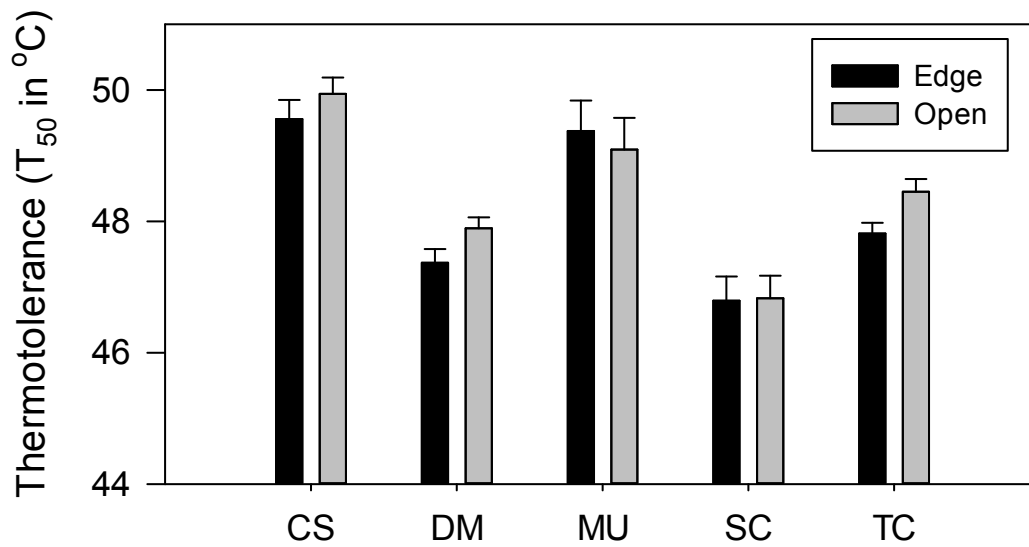


Figure 4: Recovery of T<sub>50</sub> after 24 hours. Each point represents mean of difference between T<sub>50</sub> measured immediately after heat treatment and 24 hours after heat treatment. The error bars are 95% confidence intervals.



Figure

5: Variation in thermotolerance of individuals from open and edge habitats. Each bar represents mean of species from edge or open habitats. Black bars represent mean from edge habitat and grey bar represents the mean from open habitat for same species (n=6). Error bars are standard errors of mean.

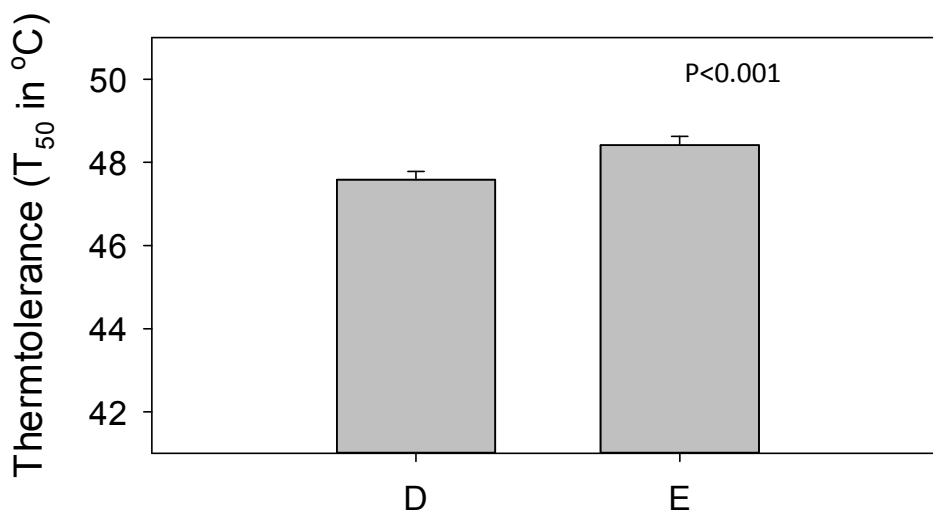


Figure 6: Variation in thermotolerance (T<sub>50</sub> of PSII function) in deciduous and evergreen tree species. Bars represents the mean of evergreen (n=10) and deciduous (n=10) species and error bar represents standard error of mean.

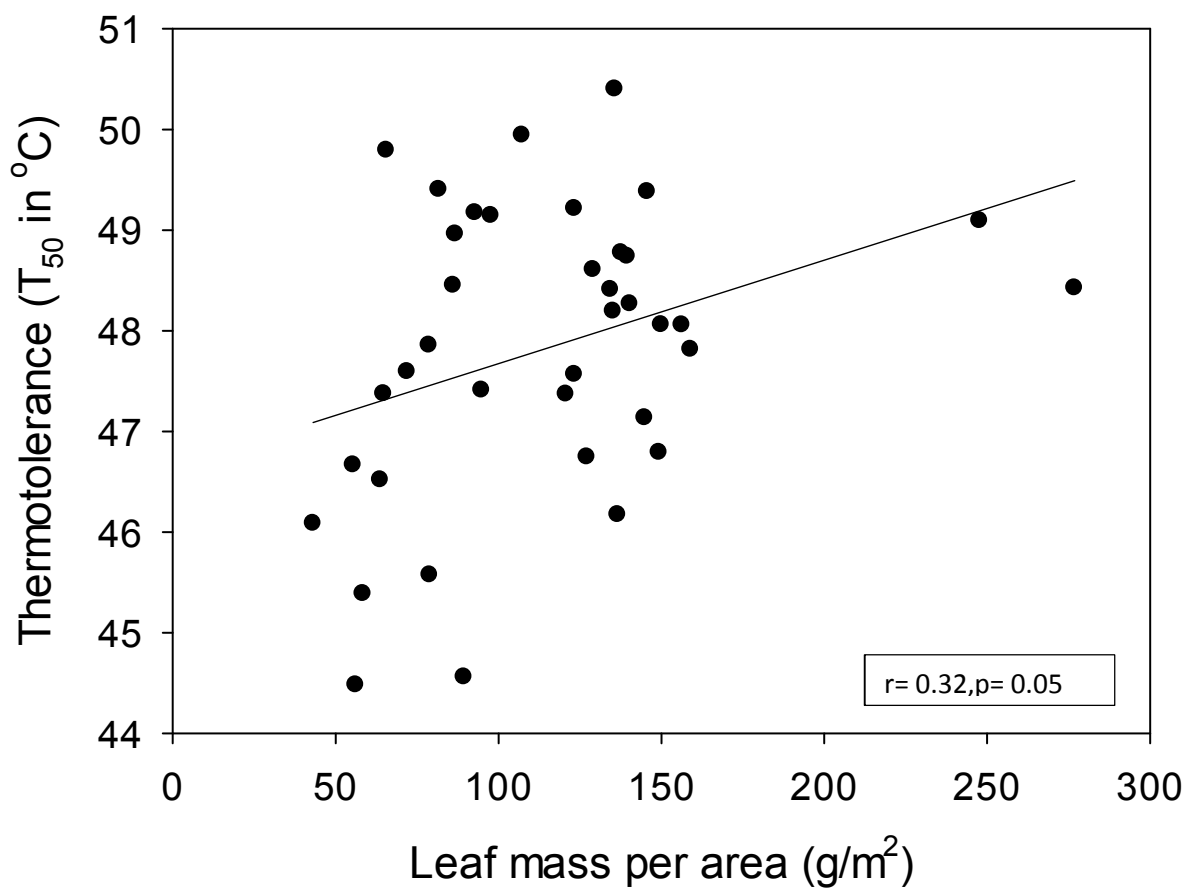


Figure 7: Relationship between leaf mass per area and thermotolerance measured as T<sub>50</sub>.

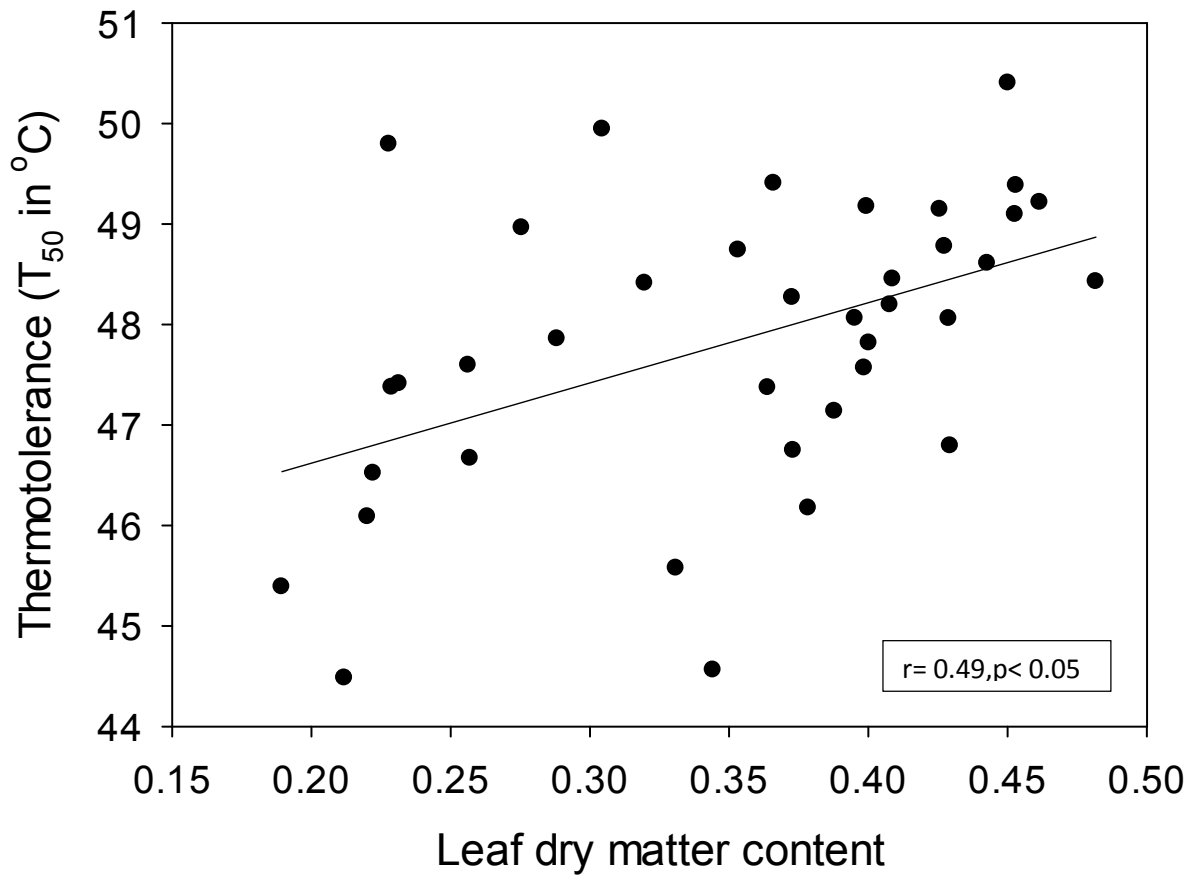


Figure 8: Relationship between leaf dry matter content and thermotolerance measured as  $T_{50}$ .

Table1: Variation in thermotolerance a) measured immediately after heat treatment ( $T_{50}$ ) and b) measured 24 hours after heat treatment ( $T_{50}$  Rec) Results of ANOVA with species nested within lifeform (shrub, liana, tree).

<b>Effect</b>	<b>DF</b>	<b>MS</b>	<b>F</b>	<b>p</b>
<b>a) Thermotolerance measured immediately after heat treatment</b>				
Species (Life form)	35	12.8	22.6	<0.001
Life form	2	8.9	15.7	<0.001
Error	192	0.6		
<b>b) Thermotolerance measured 24hours after heat treatment</b>				
Species (Life form)	35	12.0	15.7	<0.001
Life form	2	6.8	8.9	<0.001
Error	191	0.8		

Table2: Variation in recovery from heat damage. Results of repeated measures ANOVA examining effect of day on  $T_{50}$  value.

<b>Effect</b>	<b>DF</b>	<b>MS</b>	<b>F</b>	<b>P</b>
Species	37	23	25	<0.001
Recovery	1	0	0	0.600
Recovery*species	37	1	2	0.028
Error	189	0		

Table3: Variation in thermotolerance between habitats a) thermotolerance measured immediately after heat treatment ( $T_{50}$ ) b) thermotolerance measured 24hr heat treatment ( $T_{50}$  Rec). Results of ANOVA examining thermotolerance of species in open and edge habitat.

<b>Effect</b>	<b>DF</b>	<b>MS</b>	<b>F</b>	<b>P</b>
<b>a) thermotolerance measured immediately after heat treatment (<math>T_{50}</math>)</b>				
Species	4	17.0	28.8	<0.001
Habitat	1	1.0	1.7	0.195
Species*Habitat	4	0.4	0.7	0.579
Error	50	0.6		
<b>b) thermotolerance measured 24hr after heat treatment (<math>T_{50}</math> Rec)</b>				
Species	4	14.2	42.1	<0.001
Habitat	1	0.0	0.1	0.821
Species*Habitat	4	0.3	0.9	0.461
Error	50	0.3		

Table 4: Variation in thermotolerance between leaf habit (measured immediately after heat treatment ( $T_{50}$ )). Results of ANOVA with species nested within leaf habit (evergreen and deciduous) for trees.

<b>Effect</b>	<b>DF</b>	<b>MS</b>	<b>F</b>	<b>P</b>
<b>a) thermotolerance measured immediately after heat treatment (<math>T_{50}</math>)</b>				
Leaf habit	1	20.3	30.5	<0.001
Species (leaf habit)	18	12.5	18.8	<0.001
Error	99	0.7		
<b>b) thermotolerance measured 24hr after heat treatment (<math>T_{50}</math> Rec)</b>				
Leaf habit	1	9.1	11.4	0.001
Species (leaf habit)	18	9.7	12.2	<0.001
Error	98	0.8		



Table 5: Spearman correlation matrix for thermotolerance and leaf traits. The leaf traits are not transformed. Marked correlations are significant at  $p < 0.05$ .

	<b>LMA</b>	<b>LDMC</b>	<b>Thickness</b>	<b>Leaf area</b>	<b>T<sub>50</sub></b>	<b>T<sub>50</sub> rec</b>
<b>LMA</b>	–	<b>0.78</b>	<b>0.31</b>	-0.08	<b>0.32</b>	<b>0.35</b>
<b>LDMC</b>		–	-0.19	-0.20	<b>0.49</b>	<b>0.48</b>
<b>Thickness</b>			–	-0.15	-0.19	-0.14
<b>Leaf area</b>				–	0.02	0.03
<b>T<sub>50</sub></b>					–	<b>0.92</b>
<b>T<sub>50</sub> rec</b>						–

## Reference

- Anderson, J., Mccollum, G., & Roberts, W. (1990). High Temperature Acclimation in Pepper Leaves. *Plant Cell*, *25*(10), 1272–1274.
- Araújo, M. B., Ferri-Yáñez, F., Bozinovic, F., Marquet, P. A., Valladares, F., & Chown, S. L. (2013). Heat freezes niche evolution. *Ecology Letters*, *16*(9), 1206–1219. <https://doi.org/10.1111/ele.12155>
- Berry, J., & Bjorkman, O. (1980). Photosynthetic Response and Adaptation to Temperature in Higher Plants. *Annual Review of Plant Physiology*, *31*(1), 491–543. <https://doi.org/10.1146/annurev.pp.31.060180.002423>
- Bilger, H. W., Schreiber, U., & Lange, O. L. (1984). Determination of leaf heat resistance: comparative investigation of chlorophyll fluorescence changes and tissue necrosis methods. *Oecologia*, *63*(2), 256–262. <https://doi.org/10.1007/BF00379886>
- Buchner, O., Karadar, M., Bauer, I., & Neuner, G. (2013). A novel system for in situ determination of heat tolerance of plants: first results on alpine dwarf shrubs. *Plant Methods*, *9*(1), 7. <https://doi.org/10.1186/1746-4811-9-7>
- Cunningham, S. C., & Read, J. (2006). Foliar temperature tolerance of temperate and tropical evergreen rain forest trees of Australia. *Tree Physiology*, *26*(11), 1435–1443. <https://doi.org/10.1093/treephys/26.11.1435>
- Curtis, E. M., Leigh, A., & Rayburg, S. (2012). Relationships among leaf traits of Australian arid zone plants: alternative modes of thermal protection. *Australian Journal of Botany*, *60*(6), 471–483.
- Curtis, E. M., Gollan, J., Murray, B. R., & Leigh, A. (2016). Native microhabitats better predict tolerance to warming than latitudinal macro-climatic variables in arid-zone plants. *Journal of Biogeography*, *43*(6), 1156–1165. <https://doi.org/10.1111/jbi.12713>
- Deutsch, C. A., Tewksbury, J. J., Huey, R. B., Sheldon, K. S., Ghalambor, C. K., Haak, D. C., & Martin, P. R. (2008). Impacts of climate warming on terrestrial ectotherms across latitude. *Proceedings of the National Academy of Sciences of the United States of America*, *105*(18), 6668–6672.

<https://doi.org/10.1073/pnas.0709472105>

- Doughty, C. E. (2011). An In Situ Leaf and Branch Warming Experiment in the Amazon. *Biotropica*, 43(6), 658–665. <https://doi.org/10.1111/j.1744-7429.2010.00746.x>
- Doughty, C. E., & Goulden, M. L. (2009). Are tropical forests near a high temperature threshold? *Journal of Geophysical Research: Biogeosciences*, 114(1), 1–12. <https://doi.org/10.1029/2007JG000632>
- Downton, W. J. S., Berry, J. A., & Seemann, J. R. (1984). Tolerance Of Photosynthesis To High-Temperature In Desert Plants. *Plant Physiology*, 74(4), 786–790.
- Gallagher, D. 2014. Photosynthetic thermal tolerance and recovery to short duration temperature stress in desert and montane plants: a comparative study. M. Sc Thesis.
- Janzen, D. H. (1967). Why mountain passes are higher in the tropics. *The American Naturalist*, 101(919), 233-249.
- Kitao, M., Lei, T. T., Koike, T., Tobita, H., Maruyama, Y., Matsumoto, Y., & Ang, L. H. (2000). Temperature response and photoinhibition investigated by chlorophyll fluorescence measurements for four distinct species of dipterocarp trees. *Physiologia Plantarum*, 109(3), 284–290. <https://doi.org/10.1034/j.1399-3054.2000.100309.x>
- Knight, C. A., & Ackerly, D. D. (2002). An ecological and evolutionary analysis of photosynthetic thermotolerance using the temperature-dependent increase in fluorescence. *Oecologia*, 130(4), 505–514. <https://doi.org/10.1007/s00442-001-0841-0>
- Knight, C. A., & Ackerly, D. D. (2003). Evolution and plasticity of photosynthetic thermal tolerance, specific leaf area and leaf size: Congeneric species from desert and coastal environments. *New Phytologist*, 160(2), 337–347. <https://doi.org/10.1046/j.1469-8137.2003.00880.x>
- Krause, G. H., Cheesman, A. W., Winter, K., Krause, B., & Virgo, A. (2013). Thermal tolerance, net CO<sub>2</sub> exchange and growth of a tropical tree species, *Ficus*

insipida, cultivated at elevated daytime and nighttime temperatures. *Journal of Plant Physiology*, 170(9), 822–827. <https://doi.org/10.1016/j.jplph.2013.01.005>

Leigh, A., S. Sevanto, M. C. Ball, J. D. Close, D. S. Ellsworth, C. A. Knight, A. B. Nicotra, and S. Vogel.( 2012). Do thick leaves avoid thermal damage in critically low wind speeds? *New Phytologist*, **194**:477-487.

Malhi, Y., Gardner, T. a., Goldsmith, G. R., Silman, M. R., & Zelazowski, P. (2014). Tropical Forests in the Anthropocene. *Annual Review of Environment and Resources*, 39, 125–159. <https://doi.org/10.1146/annurev-environ-030713-155141>

Nathan, R., Horvitz, N., He, Y., Kuparinen, A., Schurr, F. M., & Katul, G. G. (2011). Spread of North American wind-dispersed trees in future environments. *Ecology Letters*, 14(3), 211–219. <https://doi.org/10.1111/j.1461-0248.2010.01573.x>

O'Sullivan, O. S., M. A. Heskell, P. B. Reich, M. G. Tjoelker, K. W. L. K. Weerasinghe, A. Penillard, L. Zhu, J. J. G. Egerton, K. J. Bloomfield, D. Creek, N. H. A. Bahar, K. L. Griffin, V. Hurry, P. Meir, M. H. Turnbull, and O. K. Atkin. 2017. Thermal limits of leaf metabolism across biomes. *Global Change Biology*:209-223.

Ritz, C., and J. C. Streibig.( 2005). Bioassay Analysis Using R. 2005 **12**:22.

Sastry.A unpublished (2017), Characterization of variation in thermotolerance of tropical trees from seasonally dry regions of the northern Western Ghats, PhD thesis.

Schulze, E. D. (1982). Plant life forms and their carbon, water and nutrient relations. In *Physiological plant ecology II* (pp. 615-676). Springer Berlin Heidelberg.

Sutcliffe, J. (1977). *Plants and temperature*. Edward Arnold (Publishers) Ltd.

Teskey, R., Wertin, T., Bauweraerts, I., Ameye, M., McGuire, M. A., & Steppe, K. (2015). Responses of tree species to heat waves and extreme heat events. *Plant, Cell and Environment*, 38(9), 1699–1712. <https://doi.org/10.1111/pce.12417>

Tewksbury, J. J., Huey, R. B., & Deutsch, C. a. (2008). Putting the heat on tropical

animals. *Science*, 320(5881), 1296–1297.

<https://doi.org/10.1126/science.1159328>

Vogel, S. (2005). Living in a physical world. V. Maintaining temperature. *Journal of Biosciences*, 30(5), 581–590. <https://doi.org/10.1007/BF02703556>

Weng, J. H. (2006). Underestimate of PS2 efficiency in the field due to high leaf temperature resulting from leaf clipping and its amendment. *Photosynthetica*, 44(3), 467–470. <https://doi.org/10.1007/s11099-006-0052-3>

Weng, J. H., Chen, Y. N., & Liao, T. S. (2006). Relationships between chlorophyll fluorescence parameters and photochemical reflectance index of tree species adapted to different temperature regimes. *Functional Plant Biology*, 33(3), 241–246. <https://doi.org/10.1071/FP05156>

Wright, I. J., P. B. Reich, M. Westoby, D. D. Ackerly, Z. Baruch, F. Bongers, J. Cavender-Bares, T. Chapin, J. H. C. Cornelissen, M. Diemer, J. Flexas, E. Garnier, P. K. Groom, J. Gulias, K. Hikosaka, B. B. Lamont, T. Lee, W. Lee, C. Lusk, J. J. Midgley, M. L. Navas, U. Niinemets, J. Oleksyn, N. Osada, H. Poorter, P. Poot, L. Prior, V. I. Pyankov, C. Roumet, S. C. Thomas, M. G. Tjoelker, E. J. Veneklaas, and R. Villar.(2004). The worldwide leaf economics spectrum. *Nature* **428**:821-827.

Zhang, J. L., Poorter, L., Hao, G. Y., & Cao, K. F. (2012). Photosynthetic thermotolerance of woody savanna species in China is correlated with leaf life span. *Annals of Botany*, 110(5), 1027–1033. <https://doi.org/10.1093/aob/mcs172>

## Appendix

Table1 :List of species

FAMILY	SPECIES	ACRONYM	plant type
Lauraceae	<i>Actinodaphne angustifolia</i>	AC	Tree
Vitaceae	<i>Embelia sp1</i>	AM	Liana
Rutaceae	<i>Atlantia racemosa</i>	AR	Tree
Vivianiaceae	<i>Caesaria sp.</i>	BO	Shrub
Phyllanthaceae	<i>Bridelia retusa</i>	BR	Tree
Celastraceae	<i>Gymnosporia rothiana</i>	BV	Shrub
Lecythidaceae	<i>Careya areborea</i>	CA	Tree
Acanthaceae	<i>Pleocaulus ritchei</i>	CAR	Shrub
Apocyanaceae	<i>Carissa carandas</i>	CC	Shrub
Rubiaceae	<i>Canthium diococcum</i>	CD	Tree
Euphorbiaceae	<i>Macaranga peltata</i>	CH	Tree
Lamiaceae	<i>Colebrookea oppositifolia</i>	CO	Shrub
Verbenaceae	<i>Callicarpa tomentosa</i>	CT	Tree
Lythraceae	<i>Woodfordia fruticosa</i>	DA	Shrub
Teliaceae	<i>Grewia tiliaefolia</i>	DH	Tree
Ebenaceae	<i>Diospyros montana</i>	DM	Tree
Eleaegnaceae	<i>Elaeagnus conferta</i>	EC	Liana
Salicaceae	<i>Flacourtia indica</i>	FI	Tree
Balsminaceae	<i>Impatiens sp</i>	GA	Herb
Euphorbiaceae	<i>Glochidion hohenackeri</i>	GH	Shrub
Smilacaceae	<i>Smilax ovalifolia</i>	GT	Liana
Oleaceae	<i>Jasminum malabaricum</i>	JM	Liana
Acanthaceae	<i>Carvia Callosa</i>	KA	Shrub
Thymelaeaceae	<i>Lasiosiphon eriocephalus</i>	LE	Shrub
Vitaceae	<i>Leea indica</i>	LI	Shrub
Anacardiaceae	<i>Mangifera indica</i>	MI	Tree
Euphorbiaceae	<i>Mallotus phillipensis</i>	MP	Tree
Melastomataceae	<i>Memycelon umbellatum</i>	MU	Tree
Menispermaceae	<i>Diploclisia macrocarpa</i>	NA	Liana
Oleaceae	<i>Olea dioica</i>	OD	Tree

Rubiaceae	<i>Pavetta indica</i>	PI	Shrub
Rubiaceae	<i>Randia dumetorum</i>	RD	Tree
Combretaceae	<i>Terminalia tomentosa</i>	SA	Tree
Myrtaceae	<i>Syzygium cumini</i>	SC	Tree
Compositae	<i>Seneciobombayensis</i>	SK	Herb
Combretaceae	<i>Terminalia bellerica</i>	TB	Tree
Combretaceae	<i>Terminalia chebula</i>	TC	Tree
Balsaminaceae	<i>Impatiens balsamina</i>	TD	Herb
Rhamnaceae	<i>Ziziphus sp.</i>	TH	Liana
Sapindaceae	<i>Allophyllus cobbe</i>	TP	Liana
Sapotaceae	<i>Xantolis tomentosa</i>	XT	Tree

Table 2: Pearson correlation matrix for thermotolerance and leaf traits. Marked correlations are significant at  $p < .05$ . LMA and LDMC are box cox transformed, leaf area and thickness ln transformed.

	<b>LMA whl</b>	<b>LDMC whl</b>	<b>log thickness</b>	<b>log area</b>	<b>T<sub>50</sub></b>	<b>T<sub>50</sub> rec</b>
<b>LMA whl</b>	–	<b>0.83</b>	<b>0.39</b>	-0.12	<b>0.40</b>	<b>0.43</b>
<b>LDMC whl</b>		–	-0.10	-0.09	<b>0.47</b>	<b>0.47</b>
<b>log thickness</b>			–	<b>-0.33</b>	-0.08	-0.08
<b>log area</b>				–	0.05	0.05
<b>T<sub>50</sub></b>					–	<b>0.95</b>
<b>T<sub>50</sub> rec</b>						–

Figure 1: Representation of habitats in Bhimasankar wild life sanctuary

