

**Characterization of variation in thermotolerance of tropical trees  
from seasonally dry regions of the northern Western Ghats**

A thesis submitted to a partial fulfilment  
of the requirement for the degree of  
Doctor of Philosophy by  
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## **Certificate**

Certified that the work incorporated in thesis titled ‘Characterization of variation in thermotolerance of tropical trees from seasonally dry regions of the northern Western Ghats’, submitted by Aniruddh Sastry was carried out by the candidate, under my supervision. The work presented here or any part of it has not been included in any other thesis submitted previously for the award of any degree or diploma from any other university or institution.

Dr. Deepak Barua  
Advisor

## **Declaration**

I declare that this written submission represents my ideas in my own words and where others' ideas have been included, I have adequately cited the original sources. I also declare that I have adhered to all principles of academic honesty and integrity and I have not misinterpreted or fabricated or falsified any idea/data/fact/source in my submission. I understand that violation of the above can cause disciplinary action by the institute and evoke penal action from the sources which have thus not been properly cited or from whom proper permission has not been taken when needed.

Aniruddh Sastry

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## **1. Introduction to the thesis**

Temperature is one of the most important abiotic factors that affects the function, survival and distribution of organisms. Organisms perform optimally within a range of temperatures defined as the performance breadth of the organism (Huey et al. 2012). Exposure to temperatures outside the performance breadth of organisms can lead to decreased performance, growth and reproduction, and ultimately to death. The upper thermal limits of an organism's survival plays an important role in determining species distribution (Araujo et al. 2013). Given the fundamental role of upper thermal limits of survival in determining the thermal niche of the organism, it is important to understand tolerance of organisms to high temperature extremes (hereafter, thermotolerance). This is particularly important given the current context of global warming and climate change, and has led to an increase in interest in thermotolerance in various organisms including marine algae (Thomas et al. 2012, Boyd et al. 2013, Thomas et al. 2016), insects (Deutsch et al. 2008, Hoffmann et al. 2013, Sunday et al. 2014, Kaspari et al. 2015), fish (Rummer et al. 2014), amphibians (Scheffers et al. 2014), reptiles (Scheffers et al. 2014, Bruschi et al. 2016), birds and mammals (Deutsch et al. 2008, Araujo et al. 2013, Sunday et al. 2014). However, plants in general and tropical trees in particular have received considerably less attention (Cunningham and Read 2003a, Offord 2011, Zhang et al. 2012, O'Sullivan et al. 2017).

### **1.1 Development of the field of thermotolerance in plants**

Some of the earliest studies in thermotolerance in plants were carried out in the late 19<sup>th</sup> century (Sachs 1864). There was a gradual increase in the number of studies from the early to mid-1900s and most of these studies focussed on plants from northern temperate Europe (Sapper 1935). Since the 1950s there was a marked increase in studies examining the upper thermal limits of plant temperature tolerance. These examined various plant types from moss, ferns and herbaceous plants to shrubs, lianas and trees. Additionally, these studies extended the geographic coverage of regions from which plants were examined to include Mediterranean (Lange 1961, Lange et al. 1974), the European alpine (Kjelvik 1976), and Scandinavian regions (Kjelvik 1976, Gauslaa 1984) and xeric deserts

and tropical regions (Lange 1959). Most of the studies during this period quantified necrotic damage in leaves on exposure to extreme temperatures as a measure of thermotolerance.

Around the 1970s a group of scientists in North America initiated studies on plants from Death Valley, California, one of the hottest regions in the world (Berry and Bjorkman 1980, Smillie and Gibbons 1981, Smillie and Hetherington 1983). They developed a new method of assessing thermotolerance, quantifying chlorophyll fluorescence as an estimate of photosynthetic function in leaves after exposure to extreme temperatures (Berry and Bjorkman 1980). Though some patterns of variation in thermotolerance were apparent – aquatic and shade tolerant plants had lower thermotolerance than xerophytes (Sapper 1935), more patterns became evident during this period. Savannah plants, which were adapted to dry and hot conditions, had higher thermotolerance than tropical rainforest plants (Biebl 1964). Studies on xeric plants continued in North America, and patterns began to emerge (Osmond et al. 1987, Nobel et al. 1991). Plants from hotter and drier areas were generally more thermotolerant. It was also understood that plants have higher thermotolerance during the hot-dry season as compared to the other seasons during the year. Although it was recognised that plants from hotter habitats have higher thermotolerance, the amount of variation in thermotolerance for co-existing plants from the same sites was surprising. From a seminal work that comprehensively synthesized information on variation in thermotolerance, some patterns emerged (Larcher 2003). This study concluded that perennials were more thermotolerant than annuals. Additionally, arctic herbaceous species had the lowest thermotolerance, tropical plants had the highest thermotolerance, while temperate plants lay somewhere in the middle. Aquatic plants had much lower thermotolerance than other groups of plants, while xeric plants had the highest thermotolerance for any plant group. Although most of these patterns hold true currently, there are some pitfalls in taking these generalizations as a norm. Tropical plants, and especially naturally growing woody species from the tropics were highly under-represented. The above analyses suggest patterns between regions and sites but ignore within site variation in thermotolerance. Recently, a study examined the relationship of thermotolerance with habitat temperature across 18 sites globally

(O'Sullivan et al. 2017), it was evident that there is a high variation in thermotolerance within a site. This study represents the most comprehensive examination of global patterns in thermotolerance, both from the point of geographic coverage and the total number of species examined. While this study showed that thermotolerance in these plants was negatively related to latitude and positively related to maximum habitat temperature, it was also evident from this analysis that there is a high variation in thermotolerance within a site.

The period from the early 1990s saw a change in focus in the studies that examined plant thermotolerance from examining ecological patterns to understanding the underlying physiological and molecular mechanisms of thermotolerance (Vierling and Nguyen 1992). Additionally, understanding thermotolerance of crop and model plants came into focus in these years (Bilger et al. 1984, Havaux 1992, 1993b, Yamada et al. 1996a, Yamada et al. 1996b, Weng and Lai 2005).

While majority of the focus moved to understanding mechanisms of thermotolerance, there were some studies that were interested in studying ecological patterns of thermotolerance (Knight and Ackerly 2001, Knight and Ackerly 2002, Barua et al. 2003, Knight and Ackerly 2003, Barua and Heckathorn 2004, Cunningham and Read 2006, Barua et al. 2008, Offord 2011). Recently, there has been a renewed interest in understanding patterns of thermotolerance in light of climate change related global warming (Araujo et al. 2013, O'Sullivan et al. 2017).

## **1.2 Variation in thermotolerance**

The majority of our understanding of thermotolerance of naturally occurring woody species comes from studies from the Mediterranean region, Scandinavian region, some temperate areas of Europe and North America, and Death Valley, California. Studies from other regions are limited (Figure 1). Variation in thermotolerance exists at various spatial scales in plants. For example, at the global scale it has been shown that tropical plants are more thermotolerant than plants from temperate regions (Larcher 2003). A study compared thermotolerance of four tropical trees with four temperate trees and found that tropical trees had higher thermotolerance than temperate trees (Cunningham

and Read 2006). Within the temperate region, there is a huge variation in thermotolerance and the patterns are not easily discernible. Thermotolerance of plants from the Scandinavian regions only 1°C lower than thermotolerance of plants from the tropical areas (Lange 1959, Biebl 1964, Gauslaa 1984). As a group, desert species from Death Valley in California seem to have the highest thermotolerance (Downton et al. 1984, Knight and Ackerly 2002, 2003). Within regions, the thermotolerance varies between habitats as seen in this study (Knight and Ackerly 2002, 2003), where chaparral desert species were more thermotolerant than the coastal congeners. A recent study (O'Sullivan et al. 2017) showed that thermotolerance was negatively related with latitude and positively related with habitat temperature. Apart from inter-specific variation in thermotolerance, thermotolerance also varies across ecotypes of plant species from different areas (McNaughton 1966, Karschon and Pinchas 1971, McNaughton 1973, Barua et al. 2003, Barua et al. 2008) and thermotolerance is higher in plant ecotypes from hotter regions.

Tropical regions have high average temperatures, and tropical organisms are thermal specialists with narrower thermal niches (Janzen 1967). This results in lower thermal safety margins in tropical organisms (Sunday et al. 2014), and suggests that increase in temperature due to the effects of climate change related global warming could affect tropical species more adversely than temperate species (Deutsch et al. 2008, Sunday et al. 2014). However, there are only a handful studies on thermotolerance of tropical trees when compared to studies from the temperate and alpine regions with only around 25 sites that have been examined. The geographic coverage is sparse and most of the tropical sites are in Australia (Karschon and Pinchas 1971, Cunningham and Read 2006, Offord 2011, O'Sullivan et al. 2017) with two sites (Kitao et al. 2000, Weng and Lai 2005, Chang et al. 2009) in the Indo-Malayan tropics, a site in south China (Zhang et al. 2012), six sites in the Neo-tropics (Biebl 1964, Krause et al. 2010, Krause et al. 2013, Krause et al. 2015, O'Sullivan et al. 2017), four sites in tropical Africa (Lange 1959) and Canary Islands (Larcher et al. 1991). Apart from the above, there are some studies that have examined thermotolerance in tropical species even though the sites of the study are not tropical (Yamada et al. 1996a, Yamada et al. 1996b).

Thermotolerance is highly dependent on recent growth conditions (Barua et al. 2008). Thermotolerance increases in drought stressed plants and also plants exposed to high light (Havaux 1992, Valladares and Pearcy 1997). This may result in seasonal changes in thermotolerance in naturally occurring plants where water, temperature and light conditions vary with seasons (Lange et al. 1981). It has been shown that thermotolerance was highest during the hottest times of the year for most species in the Asian and Australian tropics (Yamada et al. 1996b, Weng and Lai 2005, O'Sullivan et al. 2017), temperate regions (Hamerlynck and Knapp 1994), Mediterranean areas (Froux et al. 2004) and for desert species (Seemann et al. 1986). In seasonally dry tropics, conditions of high temperature, high light and low water occur simultaneously during the hot-dry season, and it would be important to understand how thermotolerance varies seasonally.

Thermotolerance may also vary with leaf developmental stage. Developing leaves are structurally under developed, metabolically highly active and are generally more susceptible to stress. While most studies that have examined developmental variation in thermotolerance find that mature leaves had higher thermotolerance than developing leaves (Gauslaa 1984, Jiang et al. 2006), some studies report the opposite pattern (Choinski and Gould 2010, Snider et al. 2010). Given that in dry tropical forests, leaves are flushed during the hottest-driest time of the year (Bhat 1992, Elliott et al. 2006, de Oliveira et al. 2015), it is important to understand how thermotolerance varies across leaf developmental stage and during different times of the year.

In the seasonally dry tropics, heat stress is generally accompanied by conditions of low water and high light. It is known that water stress increases plant performance at high temperatures (Havaux 1992). However extreme water deficiencies may exacerbate the temperature stress, and result in decreased plant performance (Way et al. 2013).

Thermotolerance has been shown to increase in plants exposed to high light (Havaux 1992, Valladares and Pearcy 1997). It is therefore important to examine the interactive effects of low water and high light on thermotolerance in trees from seasonally dry tropics.

From a survey of the literature on thermotolerance, it is apparent that tropics are highly under-represented. It also follows that statements made in Larcher 2003 may need to be re-examined. There seems to be very little understanding for the large degree of variation within sites in thermotolerance (O'Sullivan et al. 2017). Finally, due to the lack of consistency of the methodology used, it becomes difficult to compare across studies to make claims about general patterns. While a recent comprehensive study (O'Sullivan et al. 2017), found significant relationship between thermotolerance and habitat temperatures the relationship was shallow – for a  $\sim 30^{\circ}\text{C}$  difference in maximum habitat temperature there was a  $\sim 8^{\circ}\text{C}$  difference in thermotolerance. However, one needs to be cautious in drawing general conclusions from this study (O'Sullivan et al. 2017) as half of the sites in the study are from Australia (nine) – four in North America, one from Europe and there are only four sites from the Neo-tropics, out of which one is a high-altitude site. Hence, it would be pertinent to check if the shallow relationship and high intra-site variation can be generalized with a larger data set.

### **1.3 Thermotolerance and leaf traits**

Leaf traits are crucial in characterizing thermotolerance in plants (Groom et al. 2004, Curtis et al. 2012). It has recently been suggested that leaf traits may be key in maintaining the balance between leaf thermotolerance and photosynthetic stability over a range of temperature (Michaletz et al. 2016). Leaf mass per area (LMA) is an important leaf functional trait. There has been increasing attention given to leaf functional traits like LMA as they are important indicators of plant performance and have been shown to be a good quantitative index which allows comparisons between plant species. LMA is also an indicator of the ecological strategy of the plant (Wright et al. 2004, Diaz et al. 2016). High LMA species are generally slow growing, stress tolerant species, while low LMA species are fast growing and generally sensitive to stresses like drought and herbivory. Specifically, higher thermotolerance has been shown to be related to higher leaf thickness (Groom et al. 2004, Leigh et al. 2012), higher LMA (Gallagher 2014) and lower specific leaf area (SLA; inverse of leaf mass per area, LMA) (Charles A. Knight 2003). However, other studies find no relationship between thermotolerance and LMA (Zhang et al. 2012),

or see the opposite relationship (Godoy et al. 2011). Hence, it is not clear what the relationship between thermotolerance and LMA would be.

#### **1.4 Methodological considerations**

The tolerance of limits of higher plants to high temperatures is related to the sensitivity of the light-dependent reactions, occurring in the thylakoid membranes (Berry and Bjorkman 1980). Photosystem-II is recognized as being more sensitive than Photosystem-I to high temperatures (Berry and Bjorkman 1980, Havaux 1993a). Chlorophyll fluorescence has been used extensively to measure plant sensitivity and tolerance to high temperatures (Knight and Ackerly 2002, Barua et al. 2008, Krause et al. 2010, O'Sullivan et al. 2017). Other methods used to determine thermotolerance are leaf necrotic damage, electrolyte leakage, respiration rates and net assimilation rates. Chlorophyll fluorescence is related with necrotic damage, and so is a good indicator of irreversible damage (Bilger et al. 1984). Chlorophyll fluorescence is also the most temperature-sensitive irreversible step of photosynthesis (Krause and Santarius 1975, Berry and Bjorkman 1980).

There are two different treatment regimes used to estimate temperature tolerance. Dynamic assays entail exposing leaves to steadily increasing temperature, while static assays expose leaves to a set temperature for a fixed duration of time. In the dynamic assays, critical temperature ( $T_c$ ) is estimated as the temperature at which rapid increase of chlorophyll is induced. Responses from dynamic assays are a product of the temperature and the duration of exposure at each temperature. The compounded time into temperature exposure increases with higher critical temperatures ( $T_c$ ). The estimates of  $T_c$  result from different times of exposure to heat stress. For example, for resultant  $T_c$  measures of 45°C and 55°C, the treatment times (at a heating rate of 1°C/minute starting from 25°C) will be 20 and 30 minutes, respectively. These issues make it difficult to directly compare two species which have different  $T_c$  values. Static assays on the other hand don't have such issues and they have been shown to be good indicators of irreversible damage (Bilger et al. 1984). In these assays dark-adapted chlorophyll fluorescence ( $F_v/F_m$ ) is quantified at



every temperature. The temperature at which  $F_v/F_m$  is 50% of controls ( $T_{50}$ ) is used as an indicator of thermotolerance.

Though the gas exchange of photosynthesis is more sensitive to temperature, the effect of temperature on gas exchange is reversible. But chlorophyll fluorescence is indicative of irreversible damage to the tissue (Bilger et al. 1984). Electrolyte leakage is less sensitive than chlorophyll fluorescence and has proved difficult to standardize for comparing between different species. Necrotic damage to the leaf is a good indicator of irreversible damage (Bilger et al. 1984). However, making comparisons between species becomes difficult due to the differences in colours of healthy leaves and subjectivity in estimating damage. The ratio of dark-adapted variable fluorescence to the maximum fluorescence ( $F_v/F_m$ ) is standardized for all healthy leaves and is around 0.8 (Berry and Bjorkman 1980). This makes comparisons between different species quantitative and therefore, more straightforward (Figure 2). The temperature at which respiration breaks down are very high and may not be physiologically relevant.

Temperatures at which chlorophyll fluorescence breaks down are higher than the temperatures at which gas-exchange starts to decrease. The differential temperature response of two species may result in a lower carbon gain for the species that is more sensitive to high temperatures. I am making the assumption that there is a direct relationship between temperature responses of gas-exchange in plants to temperature responses to chlorophyll fluorescence. However, this assumption may not always be valid, and it is possible that temperature responses to gas-exchange are what determine the carbon gain, and hence may determine species distribution.

## **1.5 Climate change and effects on tropical species**

In tropical areas, there has been an unprecedented rate of increase in surface temperatures (Malhi and Wright 2004, Malhi et al. 2014). This is predicted to continue through this century and will be accompanied by longer, more severe and more frequent droughts (Niinemets 2010). Tropical species are known to have a narrow thermal niche given their evolutionary history of experiencing relatively stable temperatures (Janzen 1967, Deutsch

et al. 2008, Curtis et al. 2016). Additionally, tropical species live in habitats which are closer to their upper critical limits, and these temperature limits are within the temperatures that the tropics may see with predicted climate change (Deutsch et al. 2008). Moreover, tropical species have limited capacity to acclimate to growth temperatures (Cunningham and Read 2003b, Krause et al. 2013), which exacerbates their vulnerability to future global rises in temperature. Finally, tropical species have lower potential to migrate due to shallower latitudinal temperature gradients in tropical regions (Wright et al. 2009). The above factors make tropical species more vulnerable to variation in climatic conditions like temperature and water availability (Seddon et al. 2016). Climate change associated changes in habitat temperature may lead to shifts in species ranges, changes in community composition (Feeley et al. 2011) and changes in ecosystem structure and function (Allen et al. 2010, Mori et al. 2015). The limited understanding of thermotolerance in tropical trees coupled with the imminent threat to tropical plants from climate change related global warming, makes it important to study variation in thermotolerance in tropical trees.

Given the lack of understanding of thermotolerance in naturally occurring tropical trees, the following questions were asked: How much do tropical tree species vary in thermotolerance? Is thermotolerance related to the season, or the developmental stage of the leaf? Is thermotolerance related to plant functional types and to leaf functional traits? Is thermotolerance of tropical species affected by water availability? The vulnerability of the tropical species of this study at present temperatures and in future climate change scenarios was estimated. Additionally, the relationship between thermotolerance and habitat temperature was examined for data extracted from the literature.

## 1.6 Tables and figures

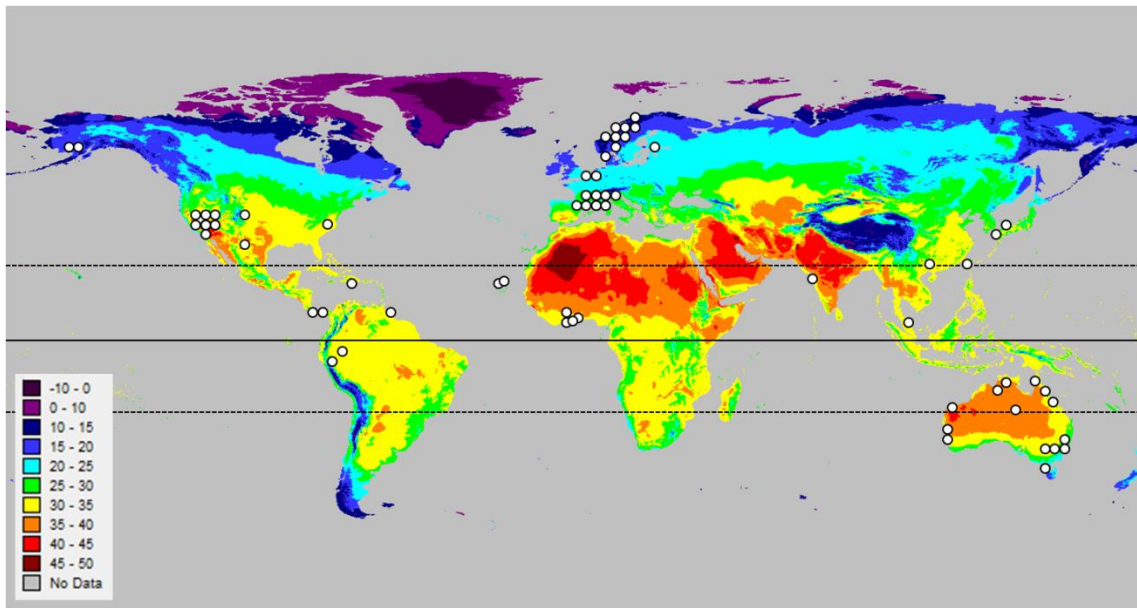


Figure 1: Global distribution of studies that have examined the upper thermal limits of naturally occurring woody plants. Each point represents a site where upper thermal limits have been examined. The colours represent the average maximum temperature of the hottest month (Hijmans et al. 2005). The solid line represents the equator, and the dotted lines represent the tropics of Cancer and Capricorn.

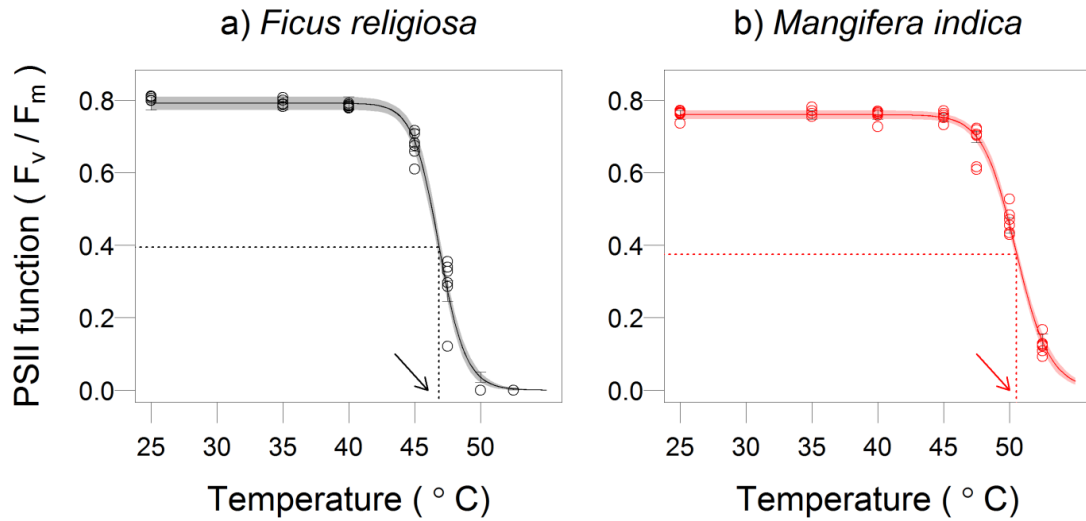


Figure 2: Representative species level temperature response curves for Photosystem II function (dark adapted  $F_v/F_m$ ). For: a) *Ficus religiosa*; b) *Mangifera indica*. Horizontal dotted lines indicate 50% of maximum values; the vertical dotted lines and arrows indicate  $T_{50}$  of PSII function - the temperature at which reduction in  $F_v/F_m$  was 50% of the maximum values.

## **2. Variation in thermotolerance of 41 tropical trees**

### **2.1 Introduction**

Geographic and taxonomic coverage of tropical plants in studies that have examined thermotolerance is sparse. Hence, our understanding of how thermotolerance varies within and between species, and the consequences of such variation is incomplete. Tropical organisms live closer to their upper critical temperatures (Janzen 1967, Deutsch et al. 2008). Thus, tropical trees may be more vulnerable to climate change associated global warming. This study characterizes patterns of variation in thermotolerance for forty-one species of tropical trees from a seasonally dry region in peninsular India.

Our understanding of thermotolerance of tropical woody species comes from around seventeen studies conducted at about twenty-five sites representing about 200 species (Table 1). From these, it is evident that the variation of thermotolerance (quantified by chlorophyll fluorescence) ranges from 34°C to 56°C (range of 22°C). For studies using more than 10 species, within site variation have been shown to be as high as 21°C (O'Sullivan et al. 2017). It is not clear why such a large variation in thermotolerance should exist within a site which experiences the same environmental conditions. It has been seen that Savannah plants of the tropics were more thermotolerant than tropical rainforest trees (Biebl 1964). For the eight species studied, tropical trees had higher thermotolerance than temperate trees (Cunningham and Read 2006). Although the highest thermotolerance for tropical trees have been known be around 56°C, there is also considerable variation in thermotolerance (Weng and Lai 2005, O'Sullivan et al. 2017).

It is known from experimental studies of plants grown under controlled conditions, that growth conditions have an effect on thermotolerance. Thermotolerance has been shown to increase with higher growth temperature (Lehel et al. 1993, Dulai et al. 1998, Haldimann and Feller 2005, Hamilton et al. 2008), high light (Havaux 1992) and when water availability is limited (Havaux 1992, Epron 1997, Ladjal et al. 2000). In naturally occurring species this may result in seasonal variation in thermotolerance that has been documented (Lange et al. 1981). Thermotolerance has been shown to be higher during

the hot-dry season when compared to the other times of the year (Lange 1961, Lange et al. 1974, Yamada et al. 1996b, Weng and Lai 2005). Given seasonal variation in thermotolerance, it was important to examine thermotolerance during different seasons to make sure that the rank order of thermotolerance of species remains the same, even though thermotolerance may change in the species.

Broad-leaved evergreen and dry-deciduous trees are important plant functional types that dominate the seasonally dry deciduous regions in the study region. These plant functional types are categorized by their leafing behaviour, but also differ in their resources acquisition strategies and tolerance to abiotic stress. Evergreen trees that maintain some portion of their canopy through the year, have a conservative resource acquisition strategy, have lower productivity, but are more resistant to drought stress (Ouédraogo et al. 2013). In contrast, deciduous trees that remain completely leafless for some duration or time through the year have a more exploitative resource acquisition strategy, but are also more susceptible to drought stress. While differences in drought tolerance has been documented between evergreen and deciduous species, not much is known about thermotolerance in these important plant functional types.

While evergreen and deciduous categories are useful and to identify important categories of plant functional types in this region, they remain discrete qualitative categories. In this study leafing behaviour - specifically average annual canopy is used to obtain a continuous and quantitative index across the range of evergreen-deciduous behaviour observed in the study species. Average annual canopy was quantified as the annual mean of monthly canopy scores. Here the most evergreen species that maintain most of their canopies will have high scores of average annual canopy near 100, and this will decreased for species that while evergreen shed a significant portion of their canopy during the dry season, and this will be the lowest for deciduous species that are leafless for some duration of the year.

Leaf mass per area (LMA) is an important leaf functional trait. There has been increasing attention given to leaf functional traits like LMA as they are important indicators of plant

performance and have been shown to be a good quantitative index which allows comparisons between plant species. LMA is also an indicator of the ecological strategy of the plant (Wright et al. 2004, Diaz et al. 2016). High LMA species are generally slow growing, stress tolerant species, while low LMA species are fast growing and generally sensitive to stresses like drought and herbivory. There are a few studies which have shown that thermotolerance is related to leaf traits (Curtis et al. 2012). In a dry sub-tropical savannah site, thermotolerance was directly related to leaf lifespan (Zhang et al. 2012). Additionally, higher thermotolerance is related to higher leaf thickness (Groom et al. 2004, Leigh et al. 2012), higher LMA (Gallagher 2014) and lower specific leaf area (SLA; inverse of leaf mass per area, LMA) (Knight and Ackerly 2003). However, studies report no relationship between thermotolerance and LMA (Zhang et al. 2012), or see the opposite relationship (Godoy et al. 2011). Hence, it is not clear what the relationship between thermotolerance and LMA would be. Further, given that larger leaf size is associated with higher leaf temperature (Little et al. 2016), one would predict that thermotolerance of tropical trees would be positively related to leaf area.

Leaf phenological events, specifically time of leaf flush has been shown to have an effect on thermotolerance of the species (Zhang et al. 2012). Species which have flushing peaks in the cool-dry season will experience the highest number of hot days through the year. Species having their flushing peaks in the hot-dry season will experience higher number of hot days during the year than those species which peak flush during the cool-wet season. One would predict that species flushing during the cool-dry season will have the highest thermotolerance, while the ones flushing during the cool-wet season will have the lowest thermotolerance.

Tropics have seen an unprecedented increase in surface temperatures since the 1970s (Malhi et al. 2014). The frequency, severity and duration of high temperature extremes have been predicted to increase. Tropical species are known to have a narrow thermal niche given their evolutionary history of experiencing relatively stable temperatures (Janzen 1967, Deutsch et al. 2008, Curtis et al. 2016). Tropical areas have higher temperatures and hence, tropical species live closer to the thermal limits of life. Tropical

species have a limited ability to migrate, given the shallow temperature gradient in the tropics – species will have to move to farther latitudes than the temperate species to reach stay within their thermal niche (Wright et al. 2009). Tropical species have a limited ability to acclimate to change in growth temperature (Cunningham and Read 2003b, Krause et al. 2013). These reasons could make tropical vulnerable to climate change related global warming (Seddon et al. 2016). And could lead to shifts in species ranges, changes in community composition (Feeley et al. 2011) and ecosystem structure and function (Allen et al. 2010, Mori et al. 2015). Thus, it is crucial to understand variation in thermotolerance for tropical trees.

Given the lack of understanding of variation in thermotolerance in tropical trees the study asked the following questions: a) Is there variation in thermotolerance in 41 co-existing species of tropical trees and how much is the variation relative to other tropical and temperate sites? b) Does thermotolerance change with season? Specifically, do rank orders change between the seasons? c) Are evergreen species more thermotolerant than deciduous species? Moreover, is the relationship of average annual canopy, which is a quantitative estimate of deciduousness, consistent with the pattern observed for the evergreen and deciduous categories? d) Is thermotolerance related to time of leaf flush? e) Is thermotolerance related to leaf functional traits, specifically, leaf mass per area and leaf area?



## **2.2 Materials and Methods**

### **Study site and species**

This work was conducted in Pune, Maharashtra, India; in the Baner-Pashan and Pashan (Panchvati) parks; National Chemical Laboratory (NCL) campus; and, Indian Institute of Science Education and Research (IISER) campus (18.541°N, 73.803°E, 560m ASL). The Baner-Pashan and Pashan (Panchvati) Parks are urban parks of ~80 ha each, and the campuses of the NCL and the IISER cover ~ 160 ha. We examined all 41-tree species commonly found in this area. Rainfall in this region is highly seasonal with greater than 90% of the annual average rainfall of 1516 mm falling between June and October (Figure 3). Average monthly minimum temperatures in January are around 11°C, while average monthly maximum temperatures in April are around 37°C. The absolute high temperature recorded in the last decade was 42.1°C. The hottest months of April and May also represent the end of the dry season and the driest and sunniest period in the year. Rainfall in this region is highly seasonal with nearly 94% of the annual average rainfall of 1516 mm falling between the months of June to October (Figure 3). Rainfall between November and May is minimal with average monthly rainfall of less than 40 mm per month. Daily mean, maximum and minimum air temperatures were obtained from the GHCN (Global Historical Climatology Network) daily Version 3.22 (Menne 2012, Menne et al. 2012). Monthly averaged precipitation (1961 – 1990), and sunshine duration were obtained from a high resolution global dataset (Mark et al. 2002).

### **Sample collection for thermotolerance assays**

Seven fully expanded and mature leaves were collected from six individuals of every species between 28<sup>th</sup> May, 2014 and 8<sup>th</sup> June 2014 (hot-dry pre-monsoon season) and between 2<sup>nd</sup> and 13<sup>th</sup> September, 2014 (cool-wet monsoon season). To control for variation in development and other factors, only the first fully expanded and mature leaves that were free of herbivory and pathogen infections were used. The leaves were placed in a paper bag, which was placed in a sealed plastic bag. A wad of rolled wet tissue was placed inside the sealed plastic bag to maintain high moisture levels. Collected leaves were transported to the lab within an hour.

### Temperature tolerance assays

We measured the temperature response of dark adapted chlorophyll *a* fluorescence, an estimate of the maximum potential quantum yield of photosystem II – PSII (Berry and Bjorkman 1980, Krause et al. 2013). Dark adapted fluorescence is the ratio of variable and maximum fluorescence,  $F_v/F_m$ , where  $F_v = (F_m - F_o) / F_m$ , and  $F_m$  and  $F_o$  are the maximum and basal fluorescence yield, respectively, for dark adapted leaves. This physiological measure is an indicator of the integrity of the photosynthetic machinery, is particularly thermolabile, and represents a sensitive indicator of photosynthetic and organismal thermotolerance (Ladjal et al. 2000, Barua et al. 2003).

Leaves discs (0.8cm radius) from 4-6 individuals of every species were used for the assays. The entire leaflet was used for species with compound leaves, where leaflet size was smaller than the leaf punch. Leaf discs were placed between two layers of muslin cloth, covered with aluminium foil and put in a sealed zip lock bag with moist tissue at the bottom to keep the bag water saturated. This was immersed in a temperature controlled refrigerated water bath (Julabo, Model F25, Seelbach, Germany) pre-set to the desired temperature (25°C, 35°C, 40°C, 45°C, 47.5°C, 50°C or 52.5°C) for 30 min. We chose 30 min exposure durations, as preliminary experiments and previous studies showed that this resulted in irreversible damage with negligible recovery after 24 hours (Curtis et al. 2014). Temperatures of dummy leaf discs (not used for further assays) were monitored with a thermocouple attached to the underside of the leaf. Preliminary trials were conducted to determine the temperature of the water bath required to maintain the desired leaf temperatures. Following the 30-min exposure to treatment temperatures, the leaf discs were allowed to dark adapt at room temperature for an additional 30 min before dark adapted chlorophyll *a* fluorescence ( $F_v/F_m$ ) was measured with a PAM 2500 fluorometer (Walz, Effeltrich, Germany).

A four parameter logistic sigmoid curve was fitted to the chlorophyll *a* fluorescence ( $F_v/F_m$ ) values across the range of temperatures examined using the R package 'drc' (Ritz and Streibig 2005). The parameters included in the model are the upper asymptote, the lower asymptote, the steepness of the curve and the point on the X-axis at which the

value on the Y-axis reduces to half of the upper asymptote. The four-parameter model with the lower asymptote set to zero was observed to generate appropriate curves. The temperature at which reduction in chlorophyll *a* fluorescence ( $F_v/F_m$ ) was 50% of the upper asymptote ( $T_{50}$ ) was estimated from these curves. We used 7 independent leaves from an individual at each of the temperatures to generate an  $F_v/F_m$  response curve from which we estimated  $T_{50}$  for that individual. This was repeated for 4-6 replicates individuals for each species.  $F_v/F_m$ -temperature response curves for representative species are shown in Figure 2.

### **Collection of leaf samples and quantification of leaf traits**

Collection of leaf samples were undertaken in 2014, between 28<sup>th</sup> May and 8<sup>th</sup> June (dry season), and between 2<sup>nd</sup> and 13<sup>th</sup> September (monsoon season). The first fully expanded, and mature leaves from the upper sun-exposed canopy that were free from visible damage from herbivory and pathogens were collected from 4-6 individuals of every species. A telescopic leaf pruner (8 m) was used to access the leaves from the canopy. Leaves were placed in sealed plastic bags with water soaked tissue paper to maintain high moisture levels. Collected leaves were transported to the lab within an hour for quantification of leaf traits and thermotolerance.

Leaf area was measured by scanning recently collected leaves with a desktop scanner, CanoScan Lide 110 (Canon, Hanoi, Vietnam) and analysing using Image J (Version 1.47, ImageJ, USA) (Schneider et al. 2012). Leaves discs were punched with a cork borer (0.8cm radius), and discs placed in paper bags in a hot-air oven at 70°C for 3-4 days till a constant dry weight was obtained. Leaf mass per area (LMA) was estimated as the ratio of dry weight of leaf discs to the surface area of fresh leaf discs, for five separate leaves each, from five replicate individuals of every species. LMA for compound leaves were quantified as the average LMA of a leaflet.

### **Phenology monitoring and estimation of deciduousness index**

Leaf phenology was monitored for 10 established and reproductively mature individuals of species from April 2014 to March 2015. For 8 species, 10 mature individuals were not

available and between 4-8 individuals were monitored (Table 14). Phenology was not monitored for 4 species due to unavailability of sufficient individuals that could be accessed through the year. Phenology censuses were conducted between the 12<sup>th</sup> and 15<sup>th</sup> of every month on the same individuals. Phenology observations were initiated 3 months before the final 12-month study duration to calibrate and fine tune visual estimates for each species. All phenology monitoring was conducted by the same observer throughout the duration of the study to avoid observer bias.

Deciduousness was scored by visual estimation of the canopy in a semi-quantitative manner from 0-100% in steps of 10, where 0 represents full canopy and no loss of leaves, and 100 represents complete leaflessness. The foliage was further partitioned into flushing, mature, and senescing leaves based on size, colour and texture of leaves. Species for which individuals lost 80% or more of their leaves (senescing leaves not considered) at any time during the year were classified as deciduous, while all other species were classified as evergreen. The monthly measures of deciduousness were averaged over the year to obtain a deciduousness index for species. This ranged from 0-100%, where zero would indicate that the species did not exhibit any leaf loss and maintained its full canopy through the year. The deciduousness index increases with increasing loss of leaves through the year.

### **Statistical analyses**

To test differences between leaf habit and season we examined variation in thermotolerance ( $T_{50}$  of PSII function) using a mixed model ANOVA with season (dry and rainy season) and leaf habit (evergreen and deciduous) as fixed effects, and species as a random effect nested within leaf habit. Next, to specifically test for seasonal changes in thermotolerance, we examined variation in the paired differences (within individuals) between dry season and rainy season thermotolerance with a mixed model ANOVA with leaf habit (evergreen and deciduous) as a fixed effect, and species as a random effect nested within leaf habit. For both of the above analyses we used the 33 species for which we had estimates of thermotolerance for both the dry and rainy seasons (as mature or healthy leaves were not available for all species in both seasons). To satisfy normality

assumptions, LMA and leaf area were log transformed, and the deciduousness index, a percentage, was converted to a proportion between 0-1 and logit transformed  $\log(y/[1 - y])$ . Relationships between the transformed leaf trait variables and thermotolerance were analyzed using Pearson's correlations, and with Spearman's rank correlations between the untransformed variables. Estimates for thermotolerance, LMA and leaf area obtained during the dry season were used for these analyses except when these were not measured during the dry season and in these cases the rainy season values were used. We also conducted these analyses separately for the dry and rainy season. All analyses were performed using Statistica (version 9.1, Statsoft, Tulsa, OK, USA).

### 2.3 Results

The temperature response curve for all the species had a similar shape (Figure 4). It was almost flat till 40°C, and then PSII function started dropping at higher temperatures. For some species, the PSII function was close to zero at 47.5°C (*Dalbergia sissoo*; Figure 4), for others it was zero at 50°C (*Albizia saman*; Figure 4) and for a few of the species PSII function did not hit zero even at 52.5°C (*Ficus benghalensis*, *Mangifera indica*; Figure 4).

Thermotolerance ( $T_{50}$  of PSII function) was different for different species – about a 6°C range from 45°C to 51°C (Table 2 and Figure 5). The mean  $T_{50}$  for all the species was 48°C. Compared to the global dataset this  $T_{50}$  was low considering the temperature this habitat experiences.

Evergreen species had higher thermotolerance ( $T_{50}$  of PSII function) than deciduous species (Table 2 and Figure 5). Thermotolerance ( $T_{50}$  of PSII function) was higher during the hot-dry season than the cool-wet season (Table 2 and Figure 5) for some of the species and thermotolerance of the others was indistinguishable between the two seasons. Out of the 33 species which were sampled during both seasons, 26 had a higher mean thermotolerance during the hot-dry season than the cool-wet season (Figure 5). Evergreen were more thermotolerant than deciduous species at both times of the year (Table 2 and Figure 5). Similarly, deciduousness was negatively related to thermotolerance (Table 3 and Figure 8). Significantly, there was a positive correlation between thermotolerance measured during the hot-dry season and thermotolerance measured in the cool-wet season (Figure 7).

Most of the species in the study had peak leaf flush during the hot-dry season, particularly during the month of April (Figure 27). Only a few evergreen species flushed earlier during December and January, while some highly deciduous species flushed during the cool-wet season. Thermotolerance was highest for species which had their peak flush during the cool-dry season, and lowest for species which had their peak flush during the cool-wet season with the species flushing in the hot-dry season having intermediate thermotolerance ( $F = 4.53$ ,  $df = 2$ ,  $p < 0.05$ ; Figure 6).

Evergreen species had higher LMA than deciduous species (Table 12 and Figure 26). There was no detectable difference in LMA between the hot-dry season than the cool-wet season (Table 12 and Figure 26). Evergreen species had higher LMA than deciduous species during both the seasons (Table 12 and Figure 26). Species had different LMA (Table 12 and Figure 26). Thermotolerance was positively related with LMA (Table 3 and Figure 8). There was no detectable relationship between thermotolerance and leaf area (Table 3).

Thermotolerance ( $T_{50}$  of PSII function), LMA and the canopy measure values were not normally distributed, so the non-parametric Kruskal-Wallis test was performed, which qualitatively were similar to the ANOVA above. As all the variables were found to be deviating from normal, Spearman's rank correlation was also performed, and the relationships were qualitatively similar to the Pearson's correlation.

## 2.4 Discussion

Thermotolerance varied between 45°C to 51°C for the forty-one-species examined. Evergreen species had higher thermotolerance than deciduous species and thermotolerance was positively related with both the canopy indices examined. Thermotolerance was higher during the hot-dry season than the cool-wet season and was also higher for species which had peak leaf flush during the cool-dry season than during the cool-wet season. Importantly, thermotolerance was positively related to LMA. Given the context of global warming, this could imply that the effects of high temperature could be directional and species with low LMA and deciduous plant functional type could be more negatively affected.

The mean thermotolerance of all species from this site, 48°C and the range between species (45°C and 51°C) were both low in comparison to the other studies from tropical sites (Table 1). The range of variation of the present study site is very small when compared to variation in thermotolerance of sites in the temperate regions (Gauslaa 1984). Maximum daily temperature experienced in the study site over the last ten years (42°C) is only three degrees lower than the thermotolerance of the most sensitive species of the study, which implies that for some of the species the upper thermal limits of many of the species is very close to the habitat air temperatures experienced.

Evergreen species had higher thermotolerance than deciduous species. Additionally, the continuous index of leafing behaviour used here, deciduousness was negatively correlated to thermotolerance. Hence, conservative water use strategy should be related to high thermotolerance. That evergreen species had higher tolerance to stress had been seen earlier for drought stress, but this may be the first study to show the similar stress tolerance of evergreen trees to high temperatures.

LMA was positively related to thermotolerance. The results are congruent with other studies on LMA and thermotolerance (Knight and Ackerly 2003, Gallagher 2014), but differs from what others have seen (Godoy et al. 2011, Zhang et al. 2012). Taken together, this may imply that the relationship between thermotolerance and LMA is



specific to plants from hot-dry regions, and the relationship may not exist in other regions. However, there have been only a handful of studies on the relationship of thermotolerance and LMA, and needs to be examined in multiple locations.

Thermotolerance was highest for species flushing leaves in the cool-dry season, intermediate for species flushing in the hot-dry season and low for species flushing in the cool-wet season. The cool-dry season occurs before the hot-dry season. And the leaves of species which flush in the cool-wet season have to experience the whole hot-dry season, which is longer time in the hot-dry season than species flushing in any other season.

The pattern of variation in thermotolerance – evergreen species are more thermotolerant than deciduous species and that high LMA species are more thermotolerant than low LMA species – suggests that the effects of climate change related global warming will not be same on all species, but some species will be more affected than others. With increases of 3-6°C predicted in the tropical areas by the end of the century (Malhi and Wright 2004, Malhi et al. 2014), there will be winners and losers. This directional effect on species could lead to a greater decline in deciduous species (Feeley et al. 2011). This could lead to directional changes in species composition in a community, changes in community dynamics and ecosystem function in seasonally dry tropics. Similarly, greater abundance of slow growing, high LMA species could slow down the rates of vegetation-atmosphere feedback, which could further exacerbate global warming.

Thermotolerance was higher or at least the same during the hot-dry season cool-wet season. Other studies on tropical plants show that thermotolerance is higher during the hotter seasons (Yamada et al. 1996b, Weng and Lai 2005). Given that thermotolerance increases with increase in ambient temperature (Lehel et al. 1993, Dulai et al. 1998, Haldimann and Feller 2005, Hamilton et al. 2008), moderate light (Krause et al. 2015) or low water (Havaux 1992, Epron 1997, Ladjal et al. 2000), it was expected that all species will be more thermotolerant during the hot-dry season. However, the ranks of thermotolerance of the species remained similar but not exactly the same. This suggests that when making inter-specific comparisons, attention needs to be paid to the conditions

under which the study is conducted. A standardized time for measurement of thermotolerance is recommended. Additionally, species differed in the degree of intra-annual variability. This shows that one should be wary of extrapolating seasonal effects of a small group of species to all species. This would also have implications on how species with differing intra-specific variation would cope with climate change related increases in temperature. Between two species with intermediate thermotolerance, the species with low intra-specific variation may be at a higher risk than the species with high intra-specific variation. As considerable intra-specific variation in thermotolerance was observed, a more detailed study on intra-specific variation on thermotolerance was carried out.

## 2.5 Tables and figures

Table 1: Details for studies<sup>9</sup> that have examined thermotolerance of tropical and sub-tropical trees. These are categorized by the methods used: a) Leaf necrotic damage (30 min exposure); b) Critical temperature ( $T_c$ ) of basal fluorescence ( $F_o$ ) rise (1°C/min heating); c)  $T_{50}$  of PSII function measured by dark adapted chlorophyll fluorescence ( $F_v/F_m$ ) (30 min exposure). Studies by Krause *et al.* 2010, Krause *et al.* 2013, and Krause *et al.* 2015 are included but the estimates of thermotolerance are not considered as the duration of heat treatment differ. Estimates for 2 species from O'Sullivan *et al.* 2017 are included in parentheses as they are exceptionally high & physiologically unrealistic.

Study	Region	No. of species	Thermotolerance (°C)
<b>a) Leaf tissue necrosis</b>			
1) Lange and Lange 1959	Ivory Coast	4	45 – 50
	Mauritania (desert)	16	49 – 56
	Mauritania (coast)	8	47 – 51
2) Biebl 1964	Puerto Rico	22	42 – 57
3) Karschon and Pinchas 1971	Multiple sites, Australia <sup>1</sup>	1	47 – 50
4) Losch 1980	Canary Islands	27	42 – 57
<b>b) Critical temperature (<math>T_c</math>) of <math>F_o</math> increase</b>			
5) Terzaghi <i>et al.</i> 1989	Central America <sup>2</sup>	7	44 – 47
6) Kitao <i>et al.</i> 2000	Malaysia	4	45 – 46
7) Weng and Lai 2005	Taiwan	10	35 – 48
8) Lin 2012	Australia (multiple sites) <sup>3</sup>	6	47 – 49
9) Zhang <i>et al.</i> 2012	Yunnan Province, China	24	43 – 47
10) O'Sullivan <i>et al.</i> 2017	Northern Territory, Australia <sup>4</sup>	5	46 – 55
	Queensland, Australia	14	37 – 49
	Andes, Peru <sup>5</sup>	13	40 – 48
	Paracou, French Guiana	21	40 – 56
	Iquitos, Peru	13	38 – 57 (65, 67)
<b>c) <math>T_{50}</math> of PSII function as measured by <math>F_v/F_m</math></b>			
11) Larcher <i>et al.</i> 1991	Tenerife, Canary Islands	2	44 – 46
12) Yamada <i>et al.</i> 1996	Okinawa, Japan <sup>6</sup>	23	44
13) Cunningham and Read 2006	Australia (multiple sites) <sup>7</sup>	4	49 – 52
14) Krause <i>et al.</i> 2010, 2013, 2015	Panama	2	NA
15) Offord 2011	Australia (multiple sites) <sup>8</sup>	7	51 – 52
16) Present study	N. Western Ghats, India	41	45 – 50

<sup>1</sup> Study examined 3 ecotypes of *Eucalyptus camaldulensis* with tropical distributions; experiments were done in plants grown in the field in a temperate location.

<sup>2</sup> Study examined multiple crop and cultivated species, some of which were woody and of tropical origin; plants were grown in controlled environmental chambers.

<sup>3</sup> Study examined 6 *Eucalyptus* species with tropical and sub-tropical distributions. Plants were grown in a common garden at Mount Anan, Australia which has a sub-humid temperate climate.

<sup>4</sup> Site is geographically in the tropics, but is described as a temperate sub-humid vegetation and climate.

<sup>5</sup> Site is geographically in the tropics, but is a high-altitude site at 3000m.

<sup>6</sup> Study examined tropical fruit trees grown in Okinawa Island, Japan. The data for only 1 species is shown here as for the others used methodology that was not comparable.

<sup>7</sup> Study examined 8 species, of which four had tropical/sub-tropical distributions. Plants were grown in a glass house where minimum temperatures were maintained above 10°C.

<sup>8</sup> Study examined 7 *Araucariaceae* species with tropical/sub-tropical distributions. Plants were grown in a botanical garden in Sydney, Australia which has a sub-humid temperate climate.

<sup>9</sup> References to the studies mentioned in Table 1 are mentioned in Supplementary material on Page 126.

Table 2: Variation in thermotolerance of 41 species: a) Variation in thermotolerance ( $T_{50}$  of PSII function). Results from a mixed model ANOVA with season (hot-dry and the cool wet rainy season) and leaf habit (evergreen and deciduous) as fixed effects and species as a random effect nested within leaf habit. b) Seasonal change in thermotolerance (dry season  $T_{50}$  - wet season  $T_{50}$ ). Results from a mixed model ANOVA with leaf habit (evergreen and deciduous) as a fixed effect and species as a random effect nested within leaf habit.

<b>Effect</b>	<b>df</b>	<b>MS</b>	<b>F</b>	<b>p</b>
<b>a) Variation in thermotolerance between leaf habit and season:</b>				
Species [Leaf Habit]	31	16.15	23.7	<0.001
Leaf Habit	1	20.43	30.0	<0.001
Season	1	78.46	115.1	<0.001
Leaf Habit x Season	1	1.28	1.9	0.172
<b>b) Variation in paired differences in thermotolerance between seasons:</b>				
Species [Leaf Habit]	31	7.786	13.78	<0.001
Leaf Habit	1	1.689	2.99	0.086

Table 3: Relationship between deciduousness index (DI, %), leaf mass per area (LMA,  $\text{g}\cdot\text{m}^{-2}$ ), leaf area (LA,  $\text{cm}^2$ ), and thermotolerance ( $^{\circ}\text{C}$ ). Values for LMA and LA were log transformed, and DI were converted to a proportion and logit transformed to meet assumptions of normality. The upper diagonal presents Pearson's coefficients (r) for the transformed variable. The lower diagonal presents Spearman's rank correlation (R) for the untransformed variables. Value in bold were significant for  $p<0.05$  - \*, and  $p<0.01$  - \*\*.

	<b>DI</b>	<b>LMA</b>	<b>LA</b>	<b>T<sub>50</sub></b>
<b>DI</b>	–	<b>-0.35*</b>	<b>0.38*</b>	<b>- 0.46**</b>
<b>LMA</b>	- 0.23	–	-0.03	<b>0.43**</b>
<b>LA</b>	<b>0.41*</b>	-0.04	–	-0.06
<b>T<sub>50</sub></b>	<b>-0.45**</b>	<b>0.43**</b>	-0.15	–

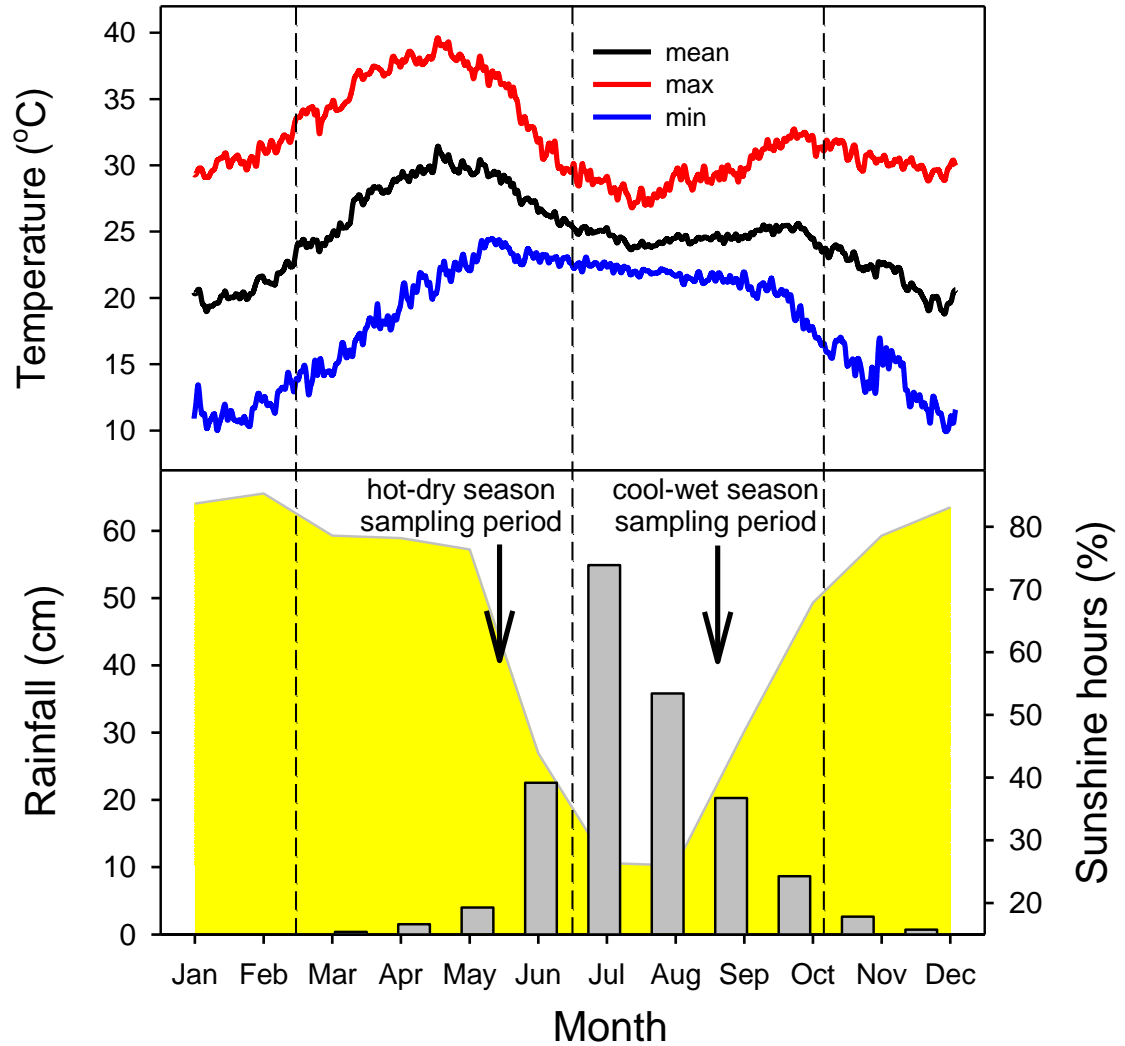


Figure 3: Climate data for the study site (Pune, Maharashtra, India). The top panel shows average daily minimum (blue), maximum (red), and mean (black) air temperatures (2005-2014). Data from GHCN (Global Historical Climatology Network) daily Version 3.22. The bottom panel presents monthly averaged precipitation (1961-1990) - grey vertical bars; and, sunshine duration (yellow curve). Precipitation and sunshine data are from a high resolution global dataset (Mark *et al.* 2002). The vertical dashed lines demarcate the three distinct seasons in the study region - hot-dry pre-monsoon (March-June), cool-wet monsoon (July-October), and cool-dry winter (November-February). Arrows indicate the hot-dry and cool-wet sampling times.

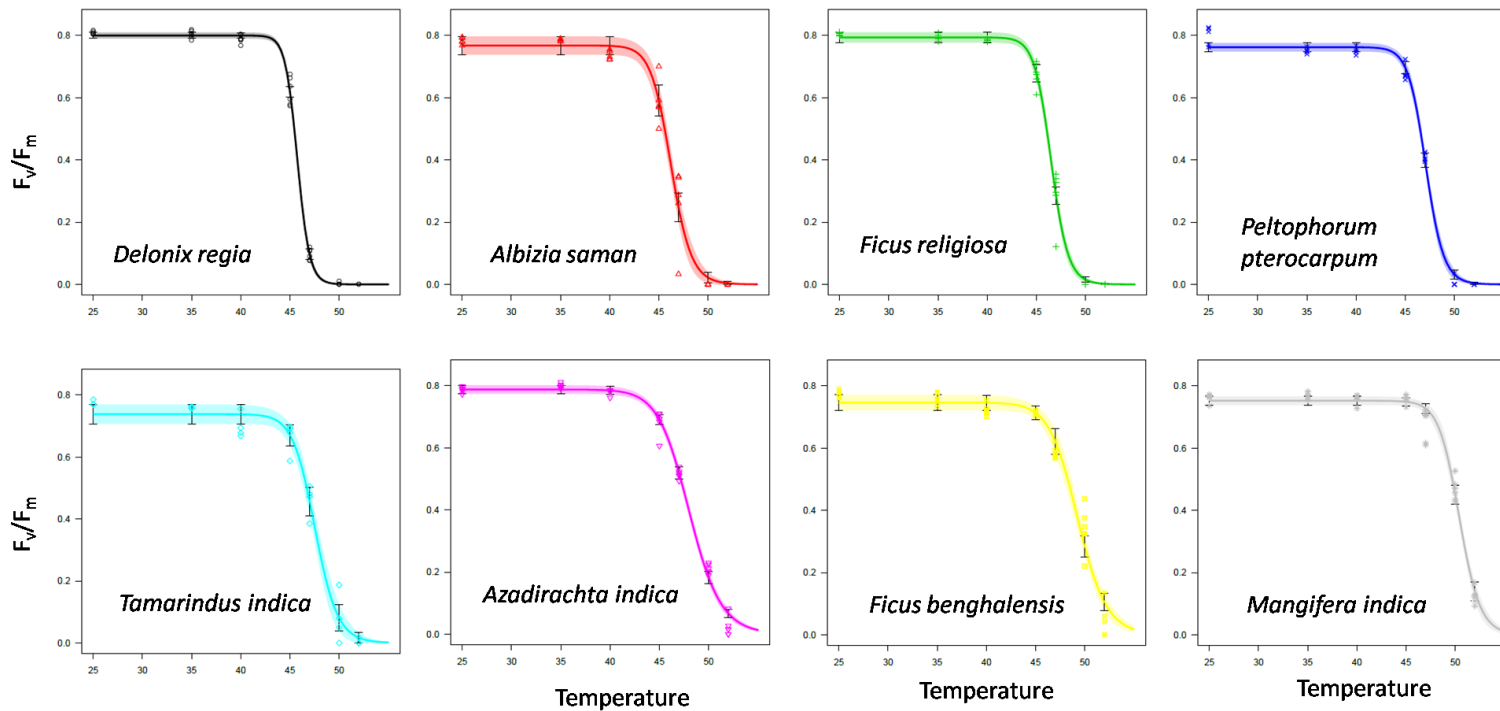


Figure 4: Representative temperature response curves spanning the entire range of thermotolerance. X-axis represents treatment temperature, while the Y-axis represents chlorophyll a fluorescence ( $F_v/F_m$ ).  $F_v/F_m$  is the ratio of variable fluorescence and maximum fluorescence, which indicates the functional efficiency of PSII in the electron transport chain. Curves were generated using the R package 'drc' (Ritz and Streibig 2005). A four-parameter logistic sigmoidal model with the lower asymptote for the set to zero was fit. The temperature at which reduction in  $F_v/F_m$  was 50% was estimated as thermotolerance ( $T_{50}$  of PSII function).



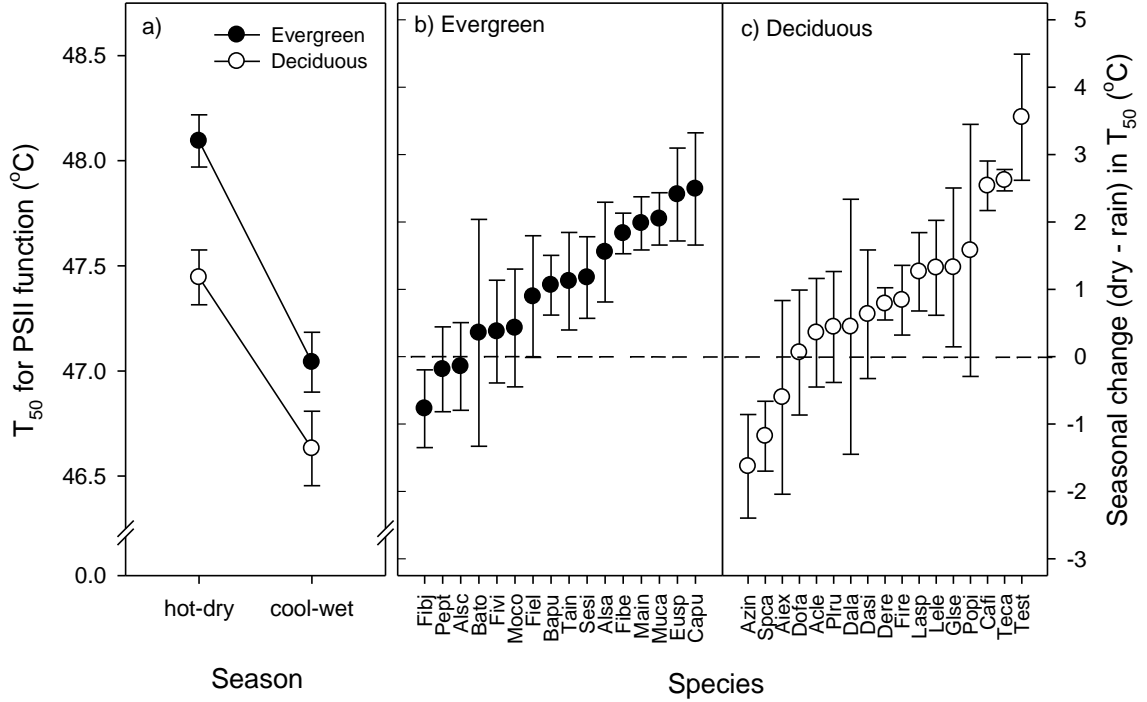


Figure 5: Variation in thermotolerance ( $T_{50}$  of PSII function). Variation in thermotolerance measured as  $T_{50}$  of PSII function ( $F_v/F_m$  - dark adapted chlorophyll  $a$  fluorescence). a) Differences between evergreen (closed circles;  $n = 16$ ) and deciduous species (open circles;  $n = 17$ ) in the hot-dry and the cool-wet rainy season. Error bars represent  $\pm$  S.E. b) and c) Change in thermotolerance from the hot-dry to the cool wet rainy season in evergreen and deciduous species respectively. The difference represented here is for paired estimates of thermotolerance in 3-6 individuals of each species. Species names are provided in Table 13. Error bars represent 95% confidence intervals.

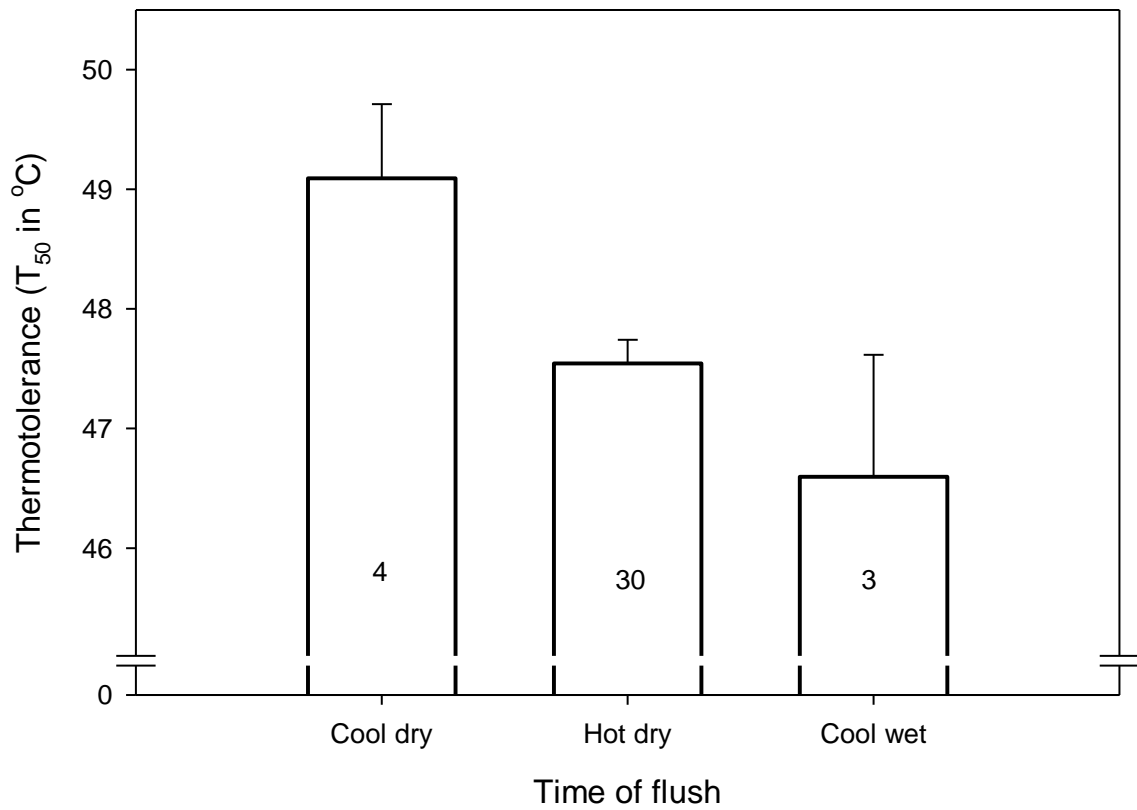


Figure 6: Variation in thermotolerance ( $T_{50}$  of PSII function) with time of peak flush. Each bar represents the average thermotolerance of species flushing during the different seasons. The number of species flushing in each season is specified by the numbers within the bars. Error bars represent standard error of mean.

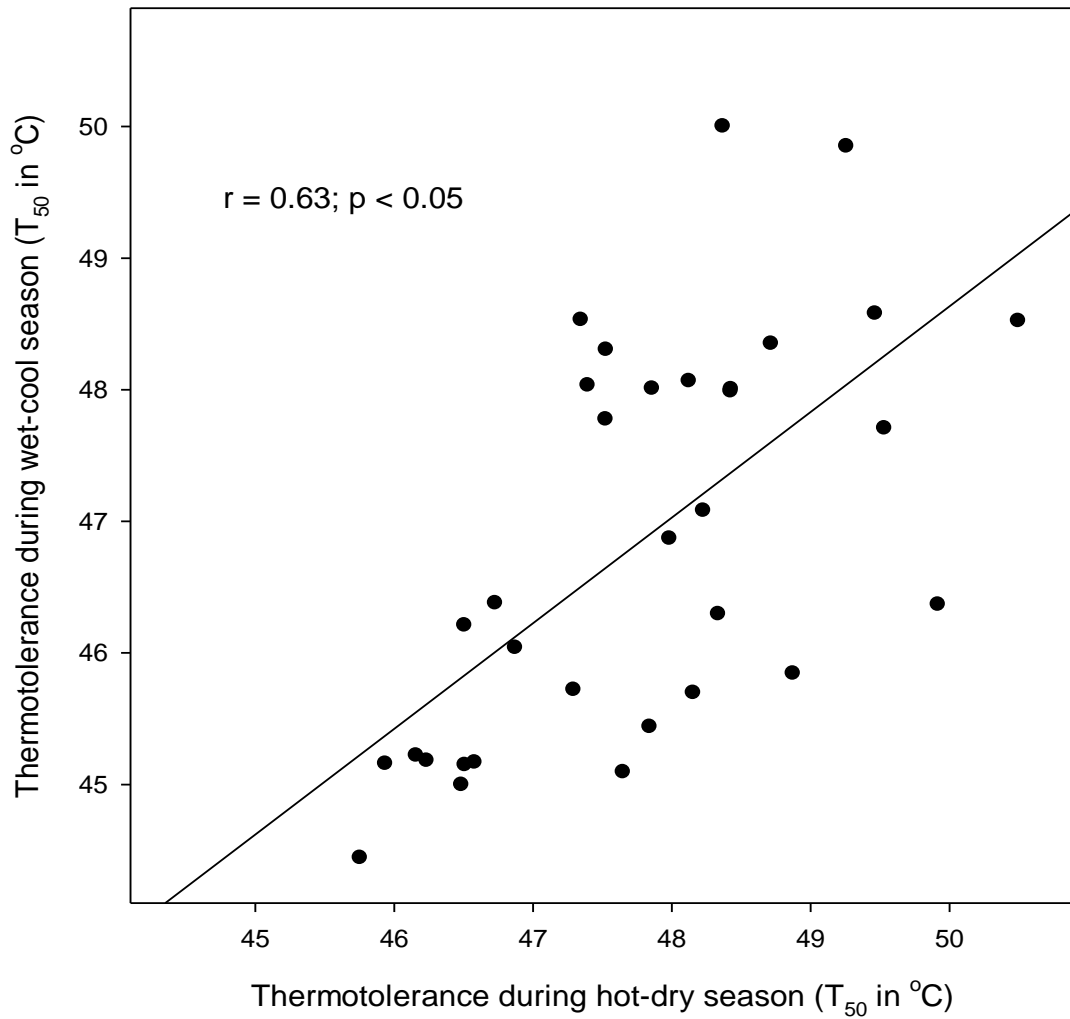


Figure 7: Relationship between thermotolerance (T<sub>50</sub> of PSII function) in the hot-dry season (X-axis) and cool-wet season (Y-axis).

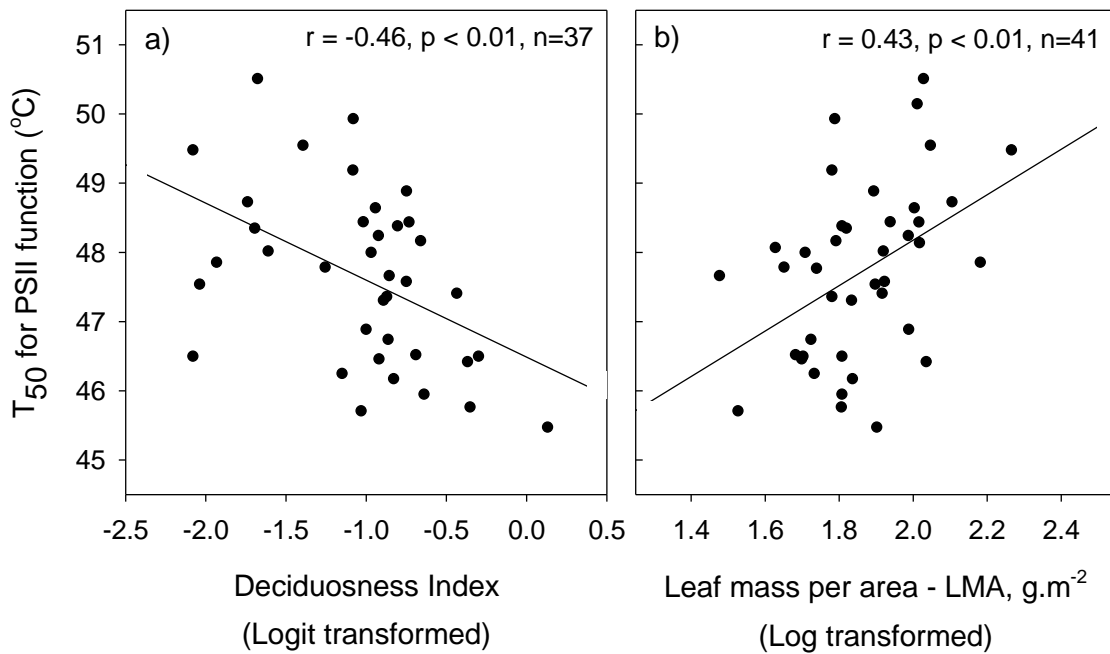


Figure 8: Relationship between thermotolerance measured as T<sub>50</sub> of PSII function ( $F_v/F_m$  - dark adapted chlorophyll *a* fluorescence) and deciduousness and LMA

a) deciduousness - this is a continuous index representative of the spectrum from evergreen species that maintain most of their canopy through the year to very deciduous species that shed all their leaves for long durations during the dry period. The values for this index were converted to a proportion and logit transformed to meet assumptions of normality. b) Leaf mass per area (LMA, g·m<sup>-2</sup>). LMA was log transformed to meet assumptions of normality.

### **3. Seasonal and leaf developmental stage variation in thermotolerance**

#### **3.1 Introduction**

Tropical organisms experience lower variability in environmental temperatures, on daily, annual and inter-annual temporal scales. Consequently, tropical organisms have a narrower temperature performance breath over which they can maintain optimum function (Janzen 1967, Deutsch et al. 2008). Moreover, tropical organisms, including plants are limited in their ability to acclimate (Cunningham and Read 2003b), but whether this includes limited acclimated tolerance to high temperature extremes (from here on, thermotolerance) is not known. This study examines variation in thermotolerance in six tropical trees through the year and at different stages of leaf development.

Apart from an organism's evolutionary history, thermotolerance is also known to be greatly influenced by environmental conditions experienced during growth and development (Gauslaa 1984). Higher growth temperature is known to increase thermotolerance. This is known from laboratory studies from Mediterranean tree species (Ghouil et al. 2003, Daas et al. 2008), temperate broad-leaved trees (Robakowski et al. 2012), conifers (Robakowski et al. 2002), one tropical tree species (Krause et al. 2013), annual herbs and crops from various regions (Gauslaa 1984, Chauhan and Senboku 1997, Yamasaki et al. 2002, Barua et al. 2003, Karim et al. 2003a, Karim et al. 2003b), and desert shrubs (Knight and Ackerly 2002). Understanding how thermotolerance varies with time of the year and growth conditions may give us an insight into the acclimation potential of different species.

Californian chaparral plants adapted to hot and dry environments were shown to have higher thermotolerance than their coastal congeners (Knight and Ackerly 2002, 2003). However, when grown in a common environment, thermotolerance of both these species is similar (Knight and Ackerly 2002), highlighting the importance of growth environment. In addition to temperature, moderate to high light (Havaux 1992) and

reduced water availability (Havaux 1992, Epron 1997, Ladjal et al. 2000) are known to confer higher thermotolerance in plants.

In naturally occurring plants water availability, light and temperature can change dramatically during the year. Such changes in environmental conditions can result in changes in thermotolerance through the different seasons (Lange et al. 1981).

Thermotolerance is highest during the hottest times of the year for plants growing in the Asian (Yamada et al. 1996b, Weng and Lai 2005) and Australian (O'Sullivan et al. 2017) tropics, temperate regions (Hamerlynck and Knapp 1994), Mediterranean areas (Froux et al. 2004) and deserts (Seemann et al. 1986).

However, in some temperate plants, thermotolerance was highest during winter (Gauslaa 1984, O'Sullivan et al. 2013, O'Sullivan et al. 2017). Cellular mechanisms to deal with high and low temperature stress may be common. For example, the changes in lipid composition in the thylakoid membranes, which is a response to low temperatures, could also confer thermotolerance in some plants (O'Sullivan et al. 2013). This phenomenon is called cross tolerance, and this could explain highest thermotolerance during the coldest time of the year.

Higher thermotolerance in the hottest times of the year suggests that this variation in thermotolerance is acclimatory. Such acclimatory responses may be particularly beneficial in highly seasonal environments like the seasonally dry tropical forests. Additionally, the ability to acclimate to changing conditions may play a key role in how plants respond to climate change. It is not known whether tropical trees from seasonally dry regions exhibit large variation in thermotolerance through the year and whether species differ in their ability to acclimate. Such differences could have important consequences for understanding differential responses of tropical trees to future warming (Malhi and Wright 2004, Malhi et al. 2014). In addition to the increase in mean temperatures, increased frequency, severity and duration of temperature extremes are predicted. Significantly, tropical species may be limited in their ability to acclimate to higher growth temperatures (Cunningham and Read 2003b, Krause et al. 2013), which

exacerbates their vulnerability to future global rises in temperature. If tropical species do indeed have limited ability to acclimate, it will make them highly vulnerable to future climate change associated global warming.

Thermotolerance also varies due to intrinsic factors like development stage or age. In plants, actively growing and developing leaf tissues are more sensitive to environmental stresses. Leaves may be more susceptible to stress early in development due to incomplete development of mechanical and physical structures and lower toughness is lower (Schultz et al. 1982). For majority of the plant species studied, thermotolerance has been shown to be lower in developing leaves than mature leaves (Gauslaa 1984, Jiang et al. 2006). However, some plants have higher thermotolerance in developing leaves as compared to mature leaves (Choinski and Gould 2010, Snider et al. 2010). Information on variation in thermotolerance across leaf developmental stages becomes especially important in seasonally dry tropics because the majority of the species flush new leaves during the hottest and driest time of the year (Bhat 1992, Elliott et al. 2006, de Oliveira et al. 2015). Developing during the hottest time of the year could be an extra-ordinary challenge for actively growing tissue with limited ability for transpirational cooling. Thermotolerance of leaves has been shown to decrease with age (Gauslaa 1984). This is expected as all metabolic activity is reducing in the leaf as it ages and goes toward senescence. Given that leaves have lower thermotolerance during developing stages and at the time of senescence, one would predict that during times of phenological activity (leaf flushing or senescing) thermotolerance of the species will be lower than at other times.

Given the lack of information on intra-specific variation in thermotolerance in naturally occurring tropical tree species, this study asked the following: a) If thermotolerance varied through the year and if the variation was related to seasonal variation in environmental conditions experienced. b) If thermotolerance was related to phenological events and phenological activity of the species. c) If early developmental stages of leaves were more sensitive to high temperatures than mature leaves. This study was conducted along with a larger study examining the inter-specific variation in thermotolerance for

forty-one species. From the study on species from the same area, it was shown that thermotolerance was highest during the hot-dry season (Chapter 2). So, in understanding inter-specific variation in thermotolerance, it becomes important to know the time of the year and growth conditions under which the plants were grown. This also made it essential to examine the intra-specific variation in thermotolerance throughout the year and at different stages of leaf development.



## **3.2 Materials and Methods**

### **Study site and species**

This study was conducted in the Northern Western Ghats of peninsular India; specifically, in Pune, Maharashtra, India, in the Baner-Pashan and Pashan (Panchvati) parks; and, Indian Institute of Science Education and Research (IISER) campus (18° 32' 28" N, 73°48' 11" E, 560m asl). The vegetation in the Northern Western Ghats varies from scrub/savanna to semi-evergreen forests that contain a mix of evergreen and deciduous species. We selected 6 common trees in the region that could be easily accessed through the year (Table 4). The climate is highly seasonal, and greater than 90 percent of the 1516mm annual average rainfall falls between June and October (Figure 9). The average monthly minimum temperature in January is around 11°C, and the average monthly maximum temperature in April is around 37°C. The highest daily temperature recorded in this region in last decade was 42.1°C. The hottest months of April and May also represent the end of the dry season, and the driest and sunniest period in the year. The daily mean, maximum and minimum air temperatures were obtained from the GHCN (Global Historical Climatology Network) daily Version 3.22 (Menne 2012, Menne et al. 2012). Monthly averaged precipitation (1961-1990), and sunshine duration were obtained from a high resolution global dataset (Mark et al. 2002).

### **Seasonal categories and leaf developmental stage definition**

For this study, the year was categorized into three seasons based on the distinct environmental conditions in these periods (Figure 9). The monsoonal months of July, August, September, and October had high precipitation, and low mean temperatures, and were categorized as cool and wet. The following months of November, December, January and February had negligible precipitation, and relatively low temperatures and were categorized as cool and dry. The months of March, April, May and June, were the driest and hottest months, and were categorized as hot and dry.

We examined leaves at three different developmental stages: recently unfurled but not fully expanded (immature), fully expanded but not mature (intermediate), and fully expanded and mature (mature). Leaf developmental stages were determined visually

based on size, colour, texture and toughness of leaves for each species. Collections and assays for examining variation in thermotolerance between developmental stages were undertaken when all 3 stages were present on the same individual of the species between 15 May and 15 June 2014.

### **Phenological monitoring**

Leaf phenology was monitored for 10 mature individuals of each of the 6 species from February 2014 to January 2015. Total leaf canopy, percent of flushing, mature, and senescing leaves were quantified by visual estimation on a scale from 0-100 in steps of 10, where 0 represents the complete absence of leaves (in that phenological category), and 100 indicates that all the present leaves were in that specific phenological category. Phenology censuses were conducted between 12<sup>th</sup> and 14<sup>th</sup> of every month.

### **Collection of samples, and leaf temperature tolerance assays**

Collections of leaf samples were undertaken between February 2014 and January 2015 on the 15<sup>th</sup> and 16<sup>th</sup> of every month immediately after completion of the phenology census. The first fully expanded and mature leaves from the sun-exposed canopy that were free from damage from herbivory and pathogens were collected from 3-6 individuals of every species. We targeted 6 replicate individuals, but healthy mature leaves free from herbivory and pathogens were not available for all species in all of the months. Collected leaves were placed in sealed plastic bags with water soaked tissue paper to maintain high moisture levels, and these were transported to the lab within an hour for the thermotolerance assays.

We measured the temperature response of dark adapted chlorophyll *a* fluorescence, an estimate of the maximum potential quantum yield of photosystem II (PSII). Dark adapted fluorescence is the ratio of variable and maximum fluorescence,  $F_v/F_m$ , where  $F_v = (F_m - F_o) / F_m$ , and  $F_m$  and  $F_o$  are the maximum and basal fluorescence yield, respectively, for dark adapted leaves. Leaves discs (2.5cm radius) from 3-6 individuals of every species were used for the assays. Leaf discs were placed between two layers of muslin cloth, covered with aluminium foil and put in a sealed zip lock bag with moist tissue at the bottom. This was immersed in a temperature controlled

refrigerated water bath (Julabo, Model F25, Seelbach, Germany) set to the desired temperature (25°C, 35°C, 40°C, 45°C, 47.5°C, and 50°C). The temperature of a dummy leaf discs that were not used for further assays were monitored with a thermocouple attached to the underside of the leaf. The temperature of the water bath required to maintain the desired leaf temperatures was determined by preliminary trials. After 30 min of exposure to the assay temperatures, the leaf discs were allowed to dark adapt at room temperature for another 30 min before measurement of chlorophyll *a* fluorescence ( $F_v/F_m$ ) with a PAM 2500 fluorometer (Walz, Effeltrich, Germany).

We fitted a four-parameter logistic sigmoid curve to the  $F_v/F_m$  values as a function of temperature using the R package 'drc' (Ritz and Streibig 2005). The parameters included in the model are the upper asymptote, the lower asymptote, the steepness of the curve and the point on the X-axis at which the value on the Y-axis reduces to half of the upper asymptote. The four-parameter model with the lower asymptote set to zero was observed to generate appropriate curves (Representative curves for species are shown in Figure 10). The temperature at which reduction in  $F_v/F_m$  was 50 percent of the upper asymptote ( $T_{50}$ ) was estimated from these curves. Six independent leaves from every individual were used at each of the assay temperatures to generate an  $F_v/F_m$  response curve from which we determined  $T_{50}$ . This was repeated for 3-6 replicates individuals for each species.

### **Statistical analyses**

We examined variation in  $T_{50}$  within each species through the year in a one-way ANOVA with month as a fixed effect to ask if thermotolerance varied through the year. As healthy mature leaves for all species were not available in all months we could not examine the interaction of species and month to test if variation in thermotolerance through the year was consistent across species. To do this, we examined how  $T_{50}$  varied across species for those months for which we had  $T_{50}$  estimates (February, May, June, July, August, October, and November) using a balanced factorial ANOVA with species and month as fixed effects. To test how thermotolerance differed across the three seasons in this region, we pooled  $T_{50}$  values for species within a season (as defined above) with a factorial ANOVA with species and season as fixed effects. Finally, we examined developmental

variation in thermotolerance for the six species to test how thermotolerance changes with a factorial ANOVA with species and leaf developmental stage as fixed effects. All analyses were performed using Statistica (version 9.1, Statsoft, Tulsa, OK, USA).

### 3.3 Results

*Ficus benghalensis* and *Ficus religiosa* are evergreen species and maintained some leaves throughout the year, while the rest were deciduous and were completely leafless for some duration during the study period (Figure 11 – Figure 16; lower panels). All species except *Tecoma stans* and *Terminalia catappa* had only one major flushing and senescing event in the year. *T. stans* had multiple flushing and senescing events within a year (Figure 15). *T. catappa* had a minor leaf exchange event during December – January (Figure 16). *Dalbergia sissoo* (Figure 11) and *Lagestromia speciosa* (Figure 14) appear to have two senescing events – one in February-March and the other in December-January. This is due to the fact that 2014 was a drier year than normal (El Niño year), and the leaves of some of the deciduous species senesced earlier. All the species exhibited the highest intensity of flushing during the dry season between the months of March and May. Deciduous species viz. *Dalbergia sissoo* (Figure 11), *Lagestromia speciosa* (Figure 14), *T. stans* (Figure 15) and *Terminalia catappa* exhibited the highest intensity of senescing between October and December (Figure 16). The two-evergreen species, *Ficus benghalensis* (Figure 12) and *Ficus religiosa* (Figure 13) peaked in senescence between February and May.

Temperature response curves were similar in shape for different species and during different months of the year. PSII function was stable till 40°C and then reduced at higher temperatures. Thermotolerance was different for different species ( $F=51.7$ ,  $df=5$ ,  $p<0.001$ ). Annual mean  $T_{50}$  for each species ranged from 45.96°C for *D. sissoo* to 48.72°C for *F. benghalensis*. Within a year thermotolerance varied from about 2°C in *D. sissoo* and *T. stans* to almost 4.5°C in *T. catappa* and *L. speciosa* (Table 4).

Thermotolerance for all species was highest during April to June – the hot-dry season. In general, thermotolerance was stable in months when there were mature leaves on the tree and flushing and senescing events were absent.

*D. sissoo* had relatively low thermotolerance throughout the year as compared to the other species. Thermotolerance was low in the month when the species was flushing new leaves (March), and increased a little during the other months (Figure 11). *F.*

*benghalensis* had a consistently higher thermotolerance than most species in every month. Like *D. sissoo*, *F. benghalensis* also had a lower thermotolerance during the month of peak flushing (June), and other months were indistinguishable from each other (Figure 12). *F. religiosa* had lower thermotolerance for senescing leaves, while other mature leaves had similar thermotolerance (Figure 13). There was large intra-annual variation in *L. speciosa*, with the highest thermotolerance with young leaves (May), and thermotolerance dropping off as mature leaves aged (Figure 14). *T. stans* had highly variable thermotolerance through the year with no discernible pattern. The pattern of these fluctuations seemed idiosyncratic (Figure 15). For *T. catappa*, thermotolerance was highest in June and lowest in May (Figure 16).

To examine how thermotolerance varied during the seasons, the monthly thermotolerance estimates were pooled for all the months that the estimates were available. *L. speciosa*, *T. stans* and *T. catappa* had highest thermotolerance during the hot-dry season (Table 6 and Figure 17). *F. benghalensis* remained highly thermotolerant throughout all the seasons; *D. sissoo* had consistently low thermotolerance in all seasons, while *F. religiosa* was intermediate.

Immature leaves had the lowest thermotolerance for all species and mature leaves had the highest thermotolerance for all species. Thermotolerance of intermediate stage leaves were indistinguishable from immature leaves for all species except *D. sissoo* (Table 7 and Figure 18).

### 3.4 Discussion

Thermotolerance varied for all species across the year. While there was no discernible pattern in how monthly thermotolerance varied, when examined for the different seasons thermotolerance was highest during the hot dry season for *L. speciosa* and *T. catappa* but not different for the other species. Immature leaves had lower thermotolerance than mature leaves for all the species examined. *F. benghalensis* had high thermotolerance; *D. sissoo* had low thermotolerance, while the rest of the species had intermediate thermotolerance. Intra-specific variation through the year was low for *F. benghalensis* and *D. sissoo*, and high for the rest of the species. Thermotolerance and the degree of inter-specific variation in thermotolerance could be crucial in our understanding of variation in thermotolerance in tropical trees. The species with low thermotolerance and low intra-specific variation in thermotolerance – *D. sissoo* – could be especially susceptible to climate change related global warming.

Thermotolerance varied during the different months and the pattern was not discernible. Intra-annual variation in thermotolerance ranged from 2°C in *F. benghalensis* and *D. sissoo* to more than 4°C in *L. speciosa* and *T. stans* (Table 4). The results show that there is intra-annual variation in thermotolerance for the tropical species that we studied. The degree of intra-annual variation is around the same range as other tropical species from literature (Weng and Lai 2005), but much less than Scandinavian (Gauslaa 1984) and desert plants (Lange et al. 1974). However, the intra-annual range of 2 to 4.5°C seems significant especially given that the range of thermotolerance for the 41-species studied was ~5°C. An intra-annual range of 4.5°C changes the way one interprets the 5°C difference in thermotolerance between tropical and temperate woody species (Larcher 2003) and the ~8°C range between equatorial and arctic species (O'Sullivan et al. 2017). It becomes important to take into account the time of the year and growth conditions while comparing thermotolerance across different regions and studies, as growth conditions may influence thermotolerance as much as the inter-specific differences.

Seasonally averaged thermotolerance was higher or at least the same during the hot-dry season as during the other times of the year. Thermotolerance during the hot-dry season

was higher than the other seasons for some species like *L. speciosa*, *T. stans* and *T. catappa*, while for *D. sissoo*, *F. benghalensis* and *F. religiosa* thermotolerance during the hot-dry season was no different than other seasons. Other studies on tropical plants show that thermotolerance is higher during the hotter seasons (Yamada et al. 1996b, Weng and Lai 2005). Given that thermotolerance increases with increase in ambient temperature (Lehel et al. 1993, Dulai et al. 1998, Haldimann and Feller 2005, Hamilton et al. 2008), moderate light (Havaux 1992) or low water (Havaux 1992, Epron 1997, Ladjal et al. 2000), we expected all species to be more thermotolerant during the hot-dry season. However, this was not true for all the species and species differed in the degree of inter-annual variability. This shows that one should be wary of extrapolating seasonal effects of a small group of species to all species.

Species under study could be divided into three groups – high, moderate and low thermotolerance. *F. benghalensis* had a consistently high thermotolerance throughout the year, *D. sissoo* had a consistently low thermotolerance throughout the year, while that of others was moderate (Figure 17). Another way to categorize species would be to categorize them by the degree of intra-specific variation during the year – high and low. *L. speciosa*, *T. stans* and *T. catappa* showed remarkable intra-specific variation in thermotolerance. This difference could lead to differential effects on the species. *F. benghalensis* had high thermotolerance and low intra-annual variability. *D. sissoo* had low thermotolerance and low intra-annual variability. The others had moderate thermotolerance and high intra-annual variability. This could lead to differential effects on each of these groups of species. The maximum temperature that occurs in the region is about 42°C. The PSII function of a species like *D. sissoo* could be adversely affected during the hottest days especially because it is highly sensitive to heat stress and has low intra-annual variation. While the PSII machinery of a species like *F. religiosa*, *L. speciosa* or *T. catappa* are likely to be less affected as they have moderate thermotolerance and some degree of intra-specific variation. *F. benghalensis*, on the other hand has a high thermotolerance and may not be affected even on the hottest days of the year. However, if future global warming trends continue, these species could become vulnerable to high temperatures – *F. religiosa*, *L. speciosa* or *T. catappa* on account of



moderate thermotolerance and *F. benghalensis* on account of its low intra-annual variability.

In general, thermotolerance was lower during periods of high phenological activity – flushing and senescing (Figure 11 – Figure 16). During times of no/low phenological activity thermotolerance for most species remains more or less constant. This would suggest that within mature leaves, younger leaves have lower thermotolerance than leaves that have flushed for a few months. That senescing leaves have a lower thermotolerance is not surprising. Most metabolic activity would be shutting down in the senescing leaf. This could explain the decrease in thermotolerance with age of the leaves in some species.

Immature leaves, which had just unfolded had lower thermotolerance than mature leaves. In plants actively growing and developing leaf tissues are more susceptible to environmental stresses. These results support the observation that thermotolerance is higher in mature leaves than developing leaves (Gauslaa 1984, Jiang et al. 2006). In dry tropical forests, leaves are flushed during the hottest-driest time of the year (Bhat 1992, Elliott et al. 2006, de Oliveira et al. 2015). This seems to be an adaptation for maximizing photosynthesis during times of highest sunlight and also to avoid the herbivores which are abundant during the wetter monsoon season (Murali and Sukumar 1993). Having low thermotolerance could become a major limiting step for some of the thermo-sensitive species. For example, in species like *D. sissoo*, *T. stans* and *T. catappa*, which have low thermotolerance in the developing stages leaf flushing events may get delayed if temperatures keep increasing during the hottest season. This could lead to shorter leaf lifespan for deciduous species, leading to lower productivity. Delay in leaf flushing could result in immature leaves being exposed to herbivores making them more susceptible to herbivory.

Season, leaf developmental stage and age of the leaf are not independent of each other. For example, while thermotolerance is higher during the hottest time of the year (Lange 1961, Lange et al. 1974, Lange et al. 1981, Yamada et al. 1996b, Weng and Lai 2005), it

is also lowest during developing stages of leaves (Gauslaa 1984, Jiang et al. 2006). With most of the species in dry tropical forests flushing leaves during the hottest time of the year (Bhat 1992, Elliott et al. 2006, de Oliveira et al. 2015) patterns in thermotolerance may get confounded. Hence, there is a need to standardize the time of the year and leaf developmental stage if one is to make inter-specific comparisons in thermotolerance. Given that the intra-annual variation in thermotolerance is almost as much as the inter-specific variation in thermotolerance, it would be prudent to test thermotolerance more than one time in the year. An important result of this study is that not all species have the same degree of intra-annual variation. This can have multiple implications: first, species which have low thermotolerance and cannot increase their thermotolerance during the hottest time of the year, like *D. sissoo*, may be extremely susceptible to future climate change related global warming. Second, it is not straight-forward to extrapolate the magnitude of seasonal change in thermotolerance by assessing seasonal changes in only a handful of species as was done by O'Sullivan and colleagues (O'Sullivan et al. 2017).

### 3.5 Tables and figures

Table 4: List of species examined and estimates of thermotolerance ( $T_{50}$ ). Given below are the means and annual ranges of monthly averages of  $T_{50}$  observed during the study period. Species were categorized as deciduous if species were completely leafless for some duration during the study period.

Species	Family	Leaf habit	$T_{50}$ (°C)	
			Mean	Range
<i>Dalbergia sissoo</i> DC.	Leguminosae	Deciduous	45.96	44.54 – 46.75
<i>Ficus benghalensis</i> L.	Moraceae	Evergreen	48.73	46.55 – 49.61
<i>Ficus religiosa</i> L.	Moraceae	Evergreen	46.79	44.81 – 47.76
<i>Lagerstroemia speciosa</i> (L.) Pers.	Lythraceae	Deciduous	47.22	45.47 – 49.91
<i>Tecoma stans</i> (L.) Juss. ex Kunth	Bignoniaceae	Deciduous	46.55	45.18 – 49.83
<i>Terminalia catappa</i> L.	Combretaceae	Deciduous	46.92	45.83 – 48.49

Table 5: Monthly variation in thermotolerance ( $T_{50}$ ) for each species. Results for effect of month on variation in thermotolerance from one-way ANOVAs performed separately for each species.

<b>Species</b>	<b>df</b>	<b>MS</b>	<b>F</b>	<b>p</b>
<i>D. sissoo</i>	10	2.1	6.5	<0.001
<i>F. benghalensis</i>	11	1.9	6.7	<0.001
<i>F. religiosa</i>	9	2.8	6.6	<0.001
<i>L. speciosa</i>	8	6.8	31.1	<0.001
<i>T. stans</i>	10	4.1	8.9	<0.001
<i>T. catappa</i>	9	6.3	22.9	<0.001

Table 6: Seasonal variation in thermotolerance ( $T_{50}$ ) between species. Results for an ANOVA examining average thermotolerance ( $T_{50}$ ) for species in the three distinct seasons in the study region – hot-dry (March-June), cool-wet (July-October), and cool-dry (November-February).

<b>Effect</b>	<b>df</b>	<b>MS</b>	<b>F</b>	<b>p</b>
Species	5	46.1	58.0	<0.001
Season	2	9.1	11.5	<0.001
Species X season	10	5.6	7.1	<0.001
Error	293	0.8		

Table 7: Developmental variation in thermotolerance ( $T_{50}$ ) between species. Results for an ANOVA examining variation thermotolerance ( $T_{50}$ ) between species between leaf developmental stages – immature, intermediate and mature.

<b>Effect</b>	<b>df</b>	<b>MS</b>	<b>F</b>	<b>p</b>
Species	5	12.6	153	<0.001
Developmental stage	2	21.6	261	<0.001
Species X developmental stage	10	0.3	4	<0.001
Error	36	0.1		

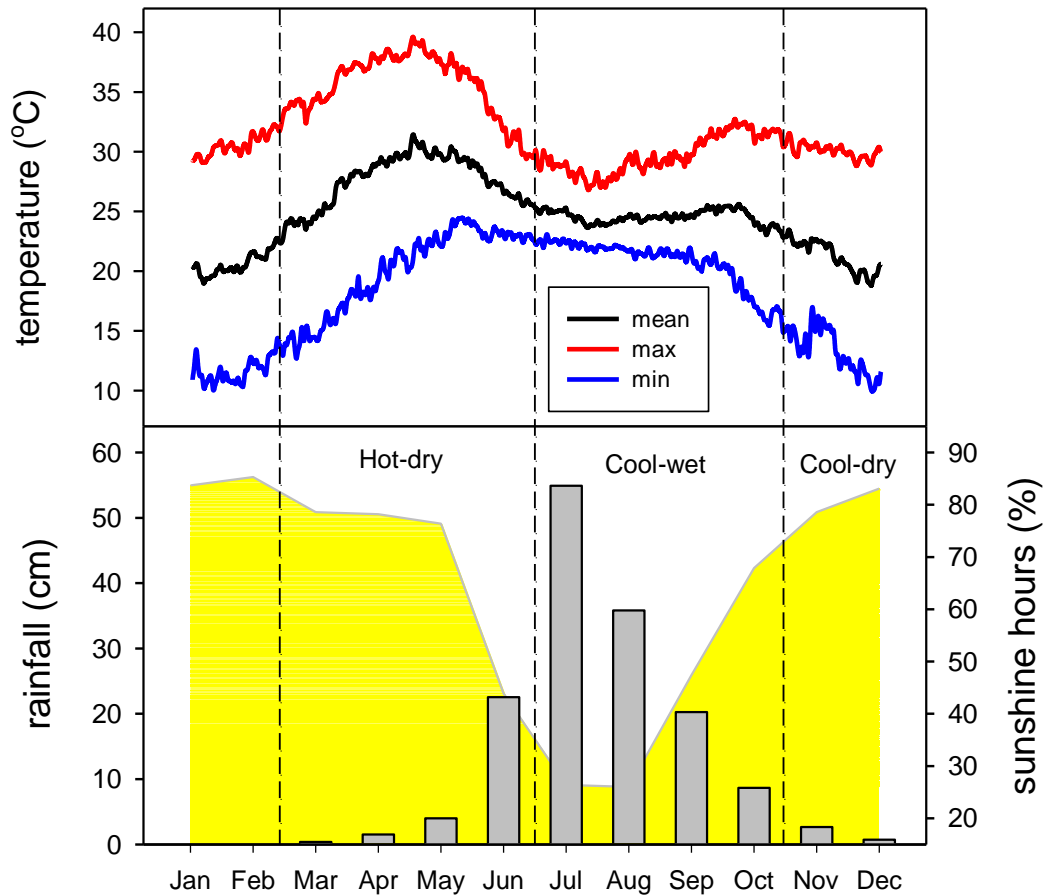


Figure 9: Climate data for the study site (Pune, Maharashtra, India). The top panel shows daily minimum (blue), maximum (red solid), and mean (black) air temperatures. The dashed red line represents the maximum daily air temperature in 2014(study period). Air temperature data were averaged for a period of ten year prior to the study (2005-2014) and were obtained from GHCN (Global Historical Climatology Network) daily Version 3.22. The bottom panel represents monthly averaged precipitation (1961-1990) - grey vertical bars; and, sunshine duration (yellow curve). Precipitation and sunshine hours data were obtained from a high resolution global dataset (Mark et al. 2002). The vertical dashed lines represent the three distinct seasons in the study region - hot-dry (March-June), cool-wet (July-October), and cool-dry (November-February).

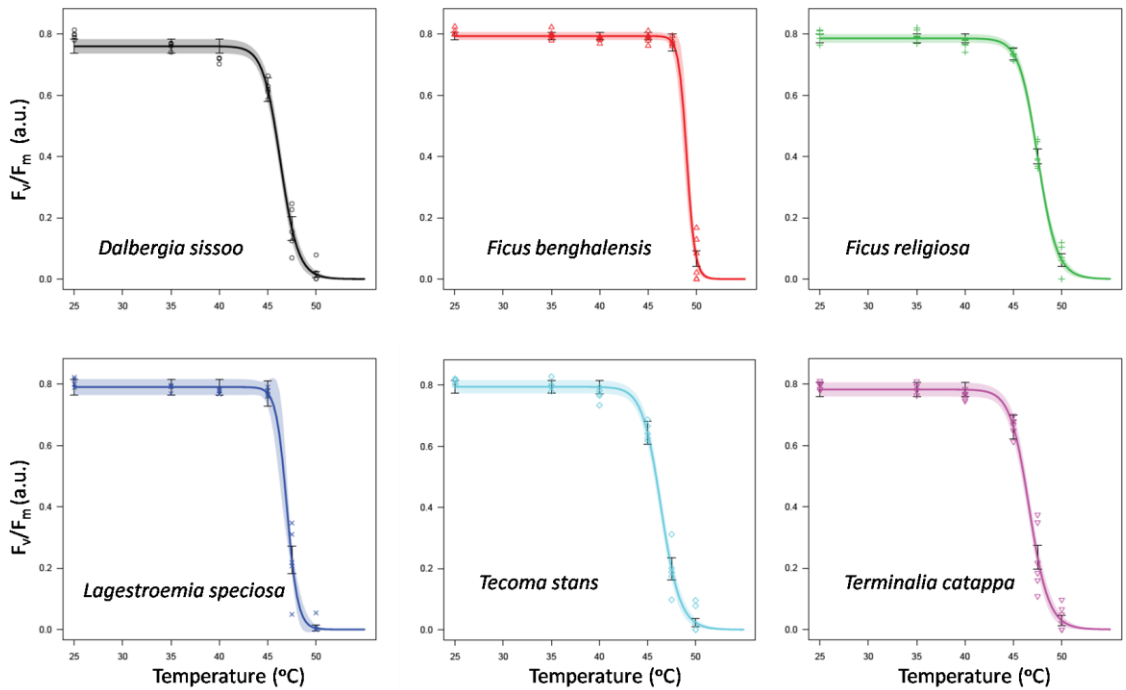


Figure 10: Representative temperature response curves for the month of August.  $F_v/F_m$  is the ratio of dark-adapted variable fluorescence and maximum fluorescence, which indicates the functional efficiency of PSII in the electron transport chain. Curves were generated using the R package drc (Ritz and Streibig 2005). The temperature at which reduction in  $F_v/F_m$  was 50% was estimated as thermotolerance ( $T_{50}$  of PSII function).



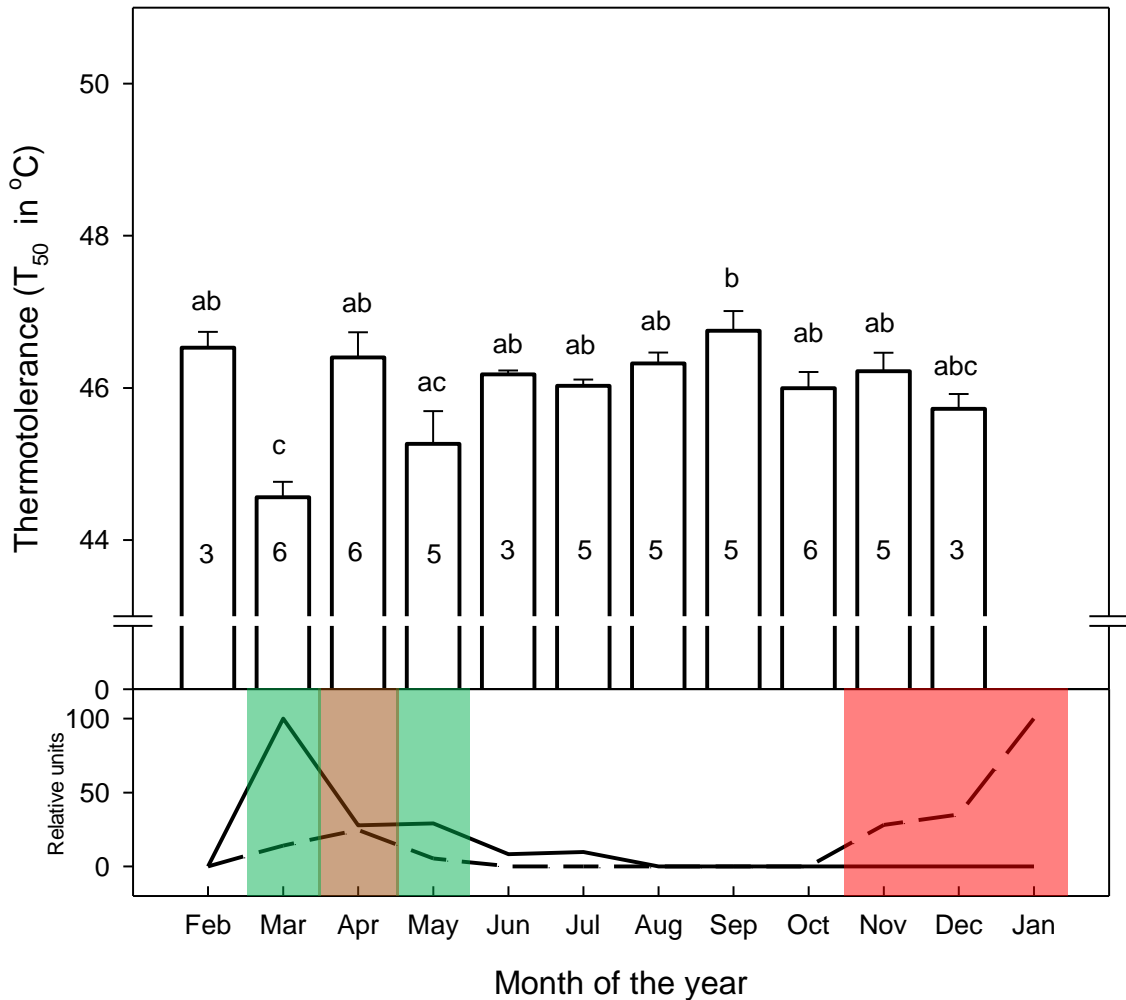


Figure 11: Monthly variation of thermotolerance ( $T_{50}$ ) in *Dalbergia sissoo* during the study period (February 2014 - January 2015). Missing data for a month indicates that healthy mature leaves were not available for that month. Error bars represent standard error of mean. The digits inside the bars are number of individuals used in each month. The lower-case letters indicate homogeneous groups based on Tukey's post-hoc test. The bottom panel shows the duration of leaf flushing (solid line) and senescing (dashed line) for the species. Periods of active leaf flushing are highlighted in green, senescing in red, and periods overlap in flushing and senescing in brown.

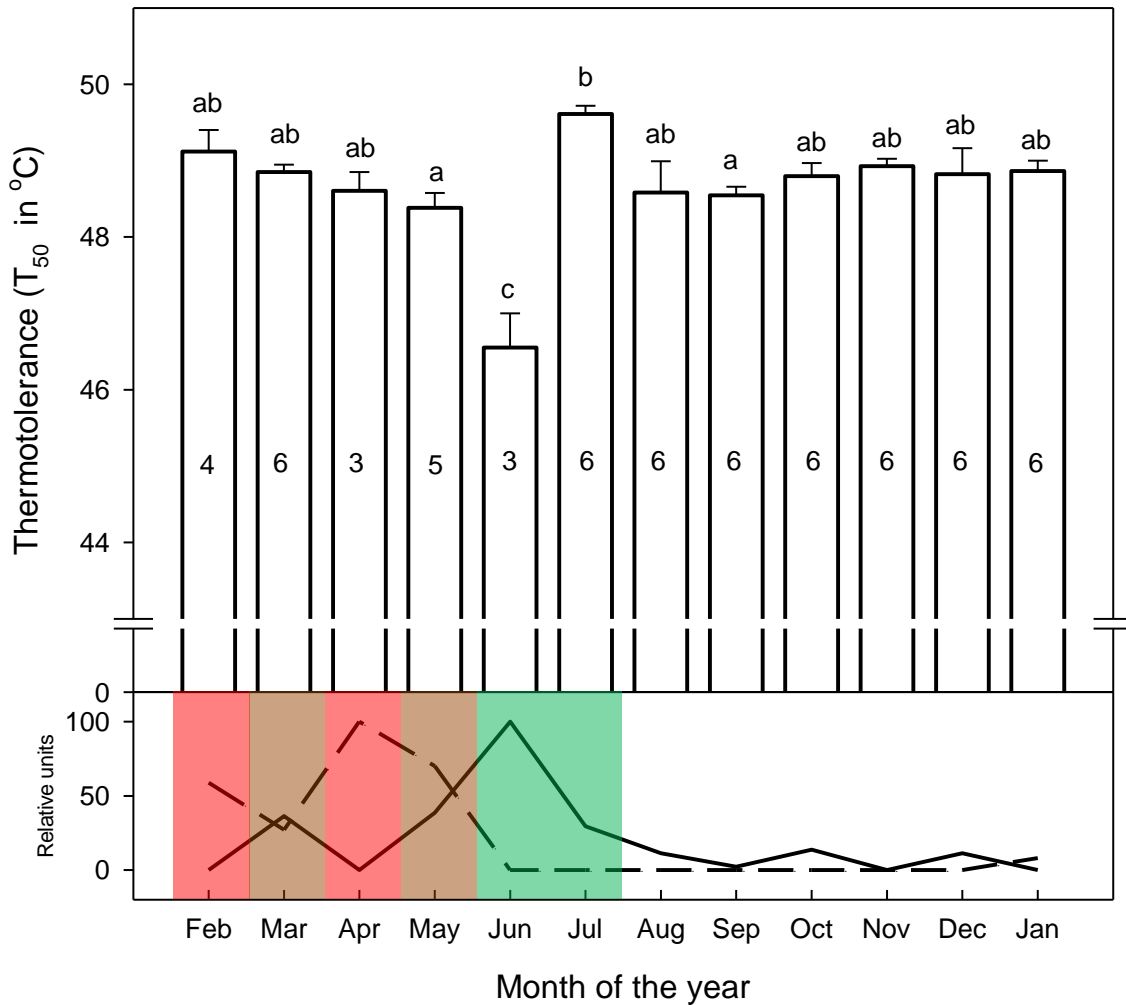


Figure 12: Monthly variation of thermotolerance ( $T_{50}$ ) in *Ficus benghalensis* during the study period (February 2014 - January 2015). Missing data for a month indicates that healthy mature leaves were not available for that month. Error bars represent standard error of mean. The digits inside the bars are number of individuals used in each month. The lower-case letters indicate homogeneous groups based on Tukey's post-hoc test. The bottom panel shows the duration of leaf flushing (solid line) and senescing (dashed line) for the species. Periods of active leaf flushing are highlighted in green, senescing in red, and periods overlap in flushing and senescing in brown.

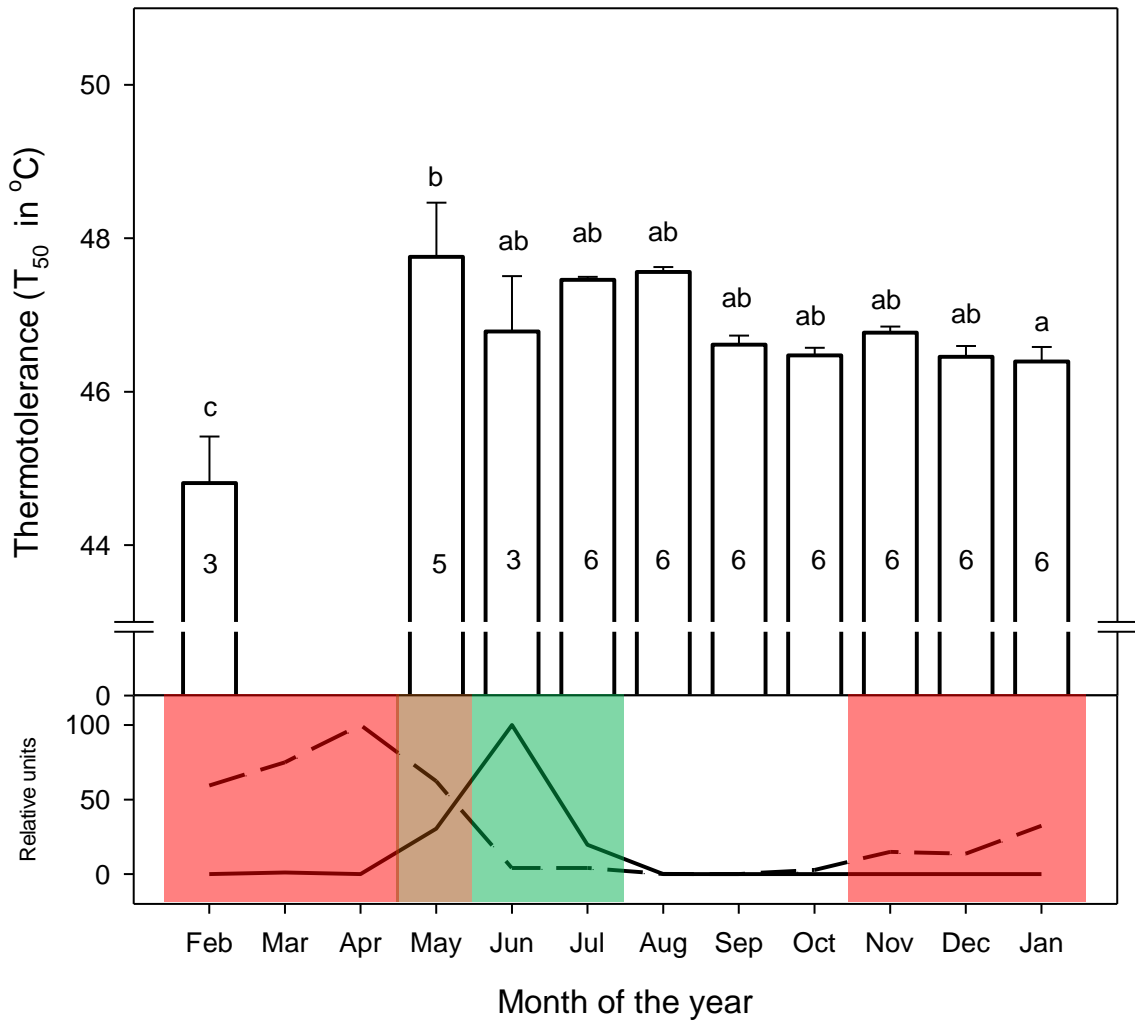


Figure 13: Monthly variation of thermotolerance ( $T_{50}$ ) in *Ficus religiosa* during the study period (February 2014 - January 2015). Missing data for a month indicates that healthy mature leaves were not available for that month. Error bars represent standard error of mean. The digits inside the bars are number of individuals used in each month. The lower-case letters indicate homogeneous groups based on Tukey's post-hoc test. The bottom panel shows the duration of leaf flushing (solid line) and senescing (dashed line) for the species. Periods of active leaf flushing are highlighted in green, senescing in red, and periods overlap in flushing and senescing in brown.

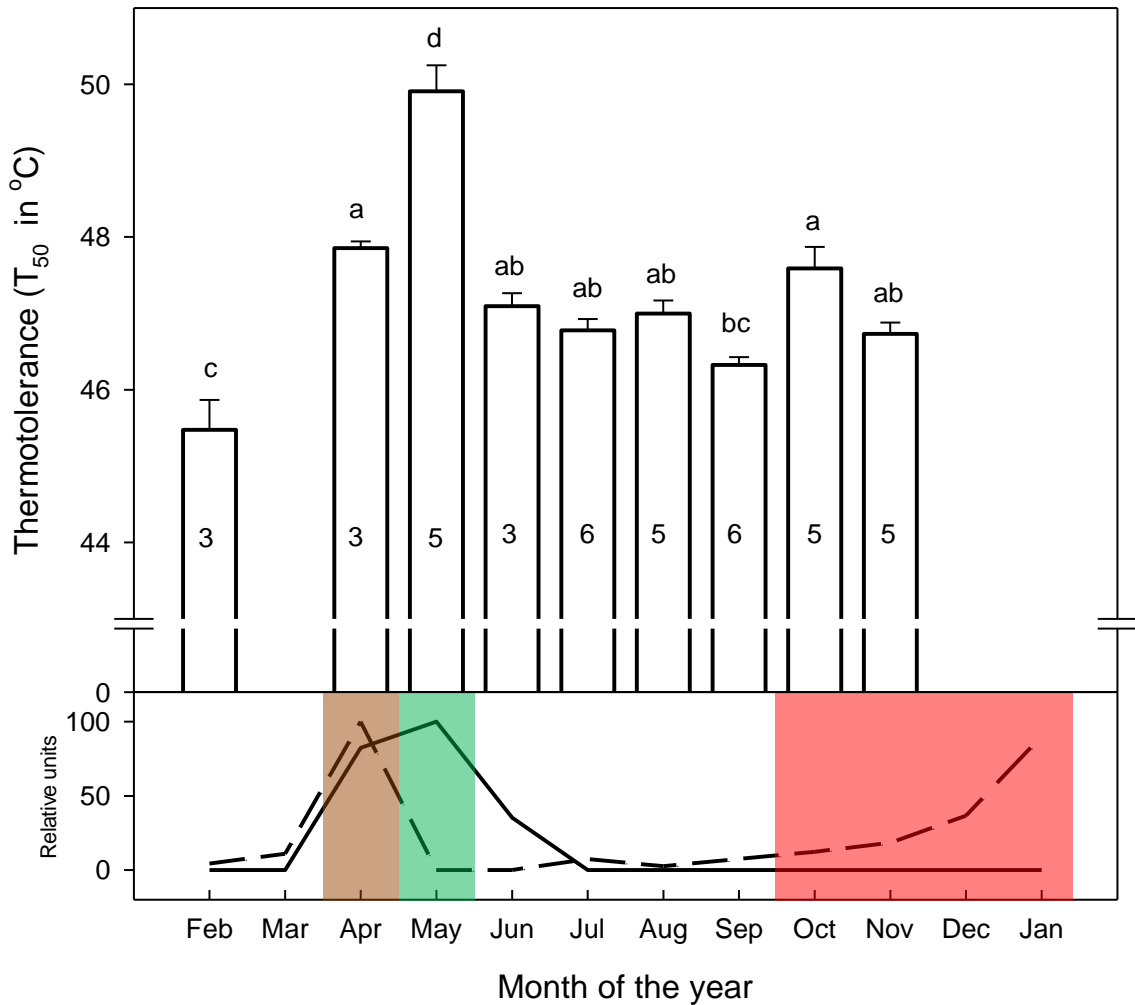


Figure 14: Monthly variation of thermotolerance ( $T_{50}$ ) in *Lagestroemia speciosa* during the study period (February 2014 - January 2015). Missing data for a month indicates that healthy mature leaves were not available for that month. Error bars represent standard error of mean. The digits inside the bars are number of individuals used in each month. The lower-case letters indicate homogeneous groups based on Tukey's post-hoc test. The bottom panel shows the duration of leaf flushing (solid line) and senescing (dashed line) for the species. Periods of active leaf flushing are highlighted in green, senescing in red, and periods overlap in flushing and senescing in brown.

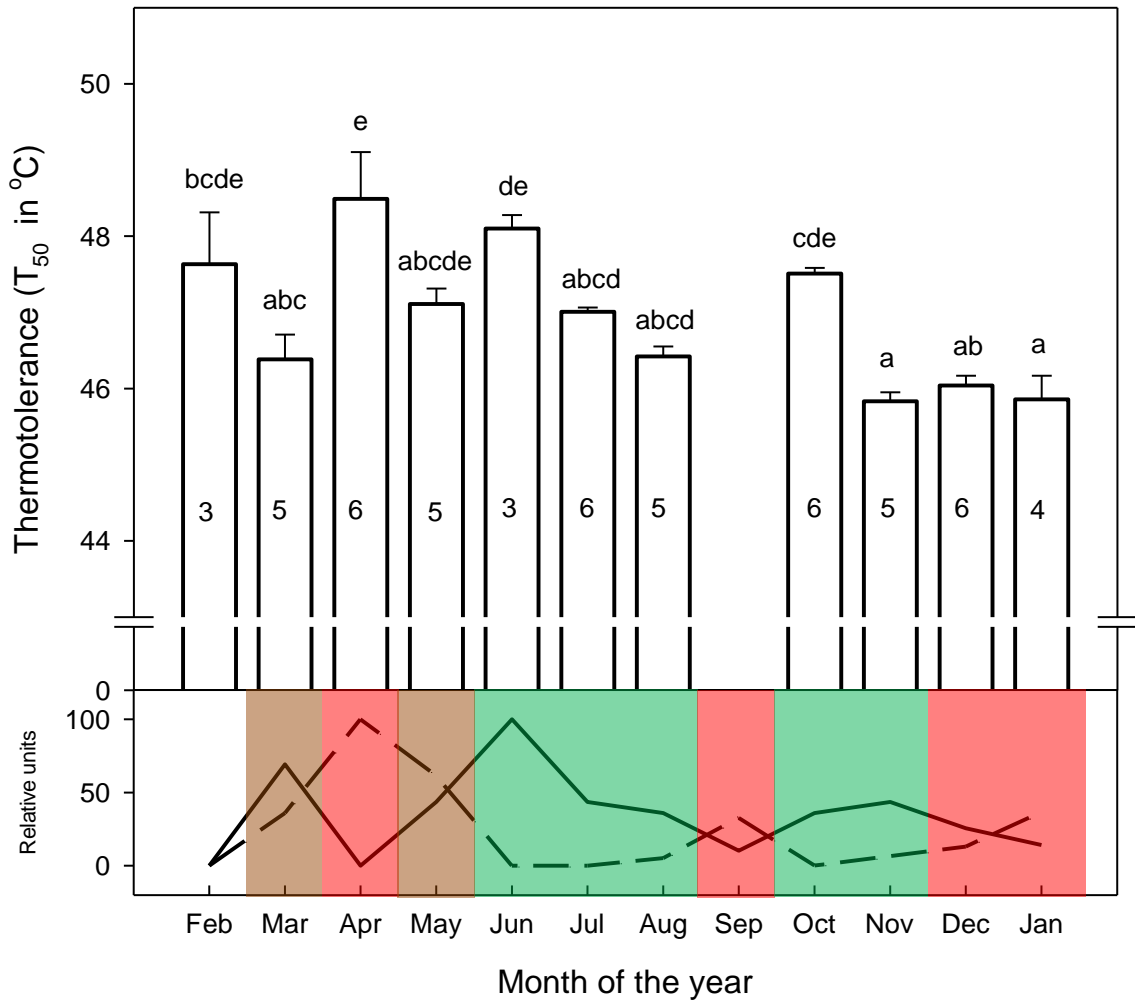


Figure 15: Monthly variation of thermotolerance ( $T_{50}$ ) in *Tecoma stans* during the study period (February 2014 - January 2015). Missing data for a month indicates that healthy mature leaves were not available for that month. Error bars represent standard error of mean. The digits inside the bars are number of individuals used in each month. The lower-case letters indicate homogeneous groups based on Tukey's post-hoc test. The bottom panel shows the duration of leaf flushing (solid line) and senescing (dashed line) for the species. Periods of active leaf flushing are highlighted in green, senescing in red, and periods overlap in flushing and senescing in brown.

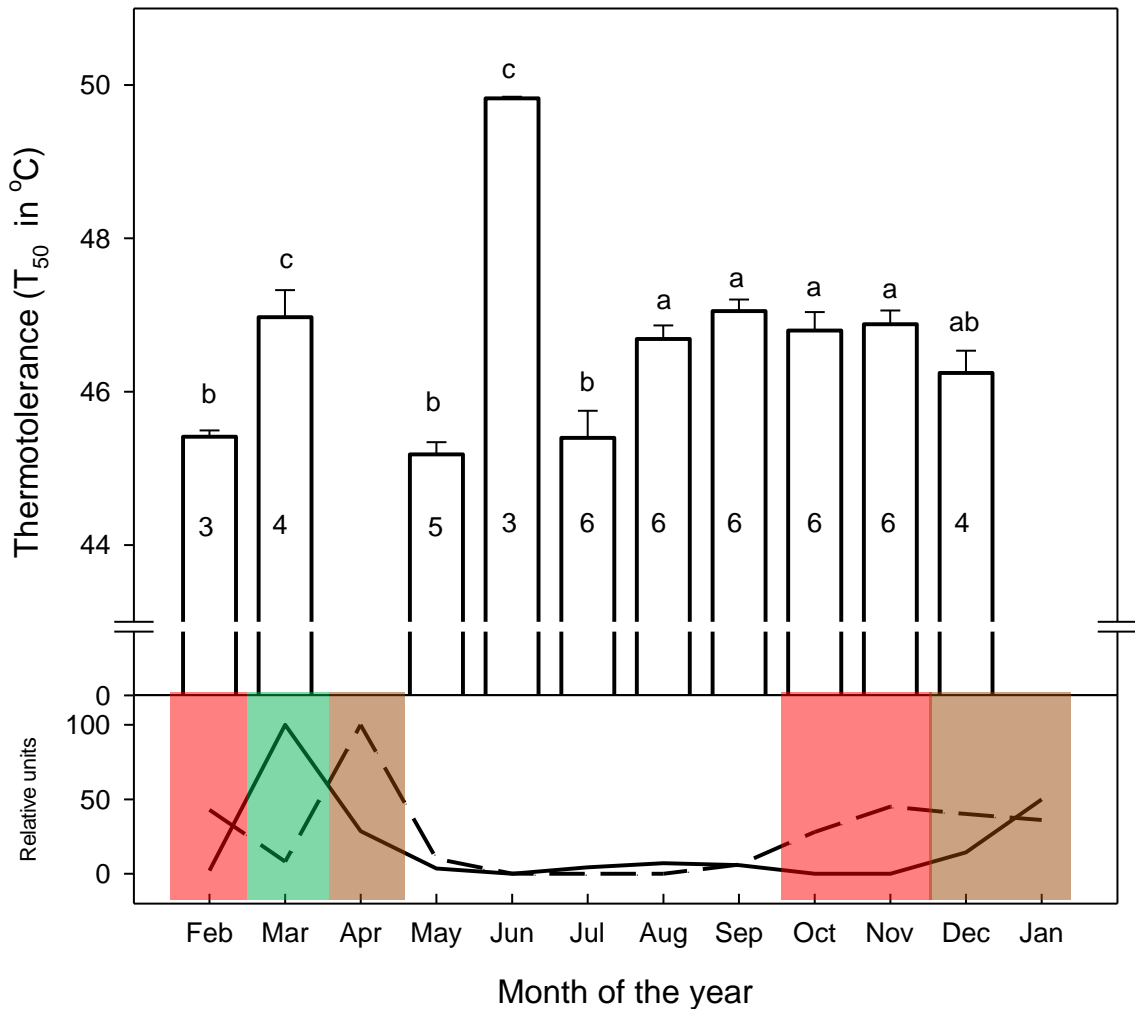


Figure 16: Monthly variation of thermotolerance ( $T_{50}$ ) in *Terminalia catappa* during the study period (February 2014 - January 2015). Missing data for a month indicates that healthy mature leaves were not available for that month. Error bars represent standard error of mean. The digits inside the bars are number of individuals used in each month. The lower-case letters indicate homogeneous groups based on Tukey's post-hoc test. The bottom panel shows the duration of leaf flushing (solid line) and senescing (dashed line) for the species. Periods of active leaf flushing are highlighted in green, senescing in red, and periods overlap in flushing and senescing in brown.

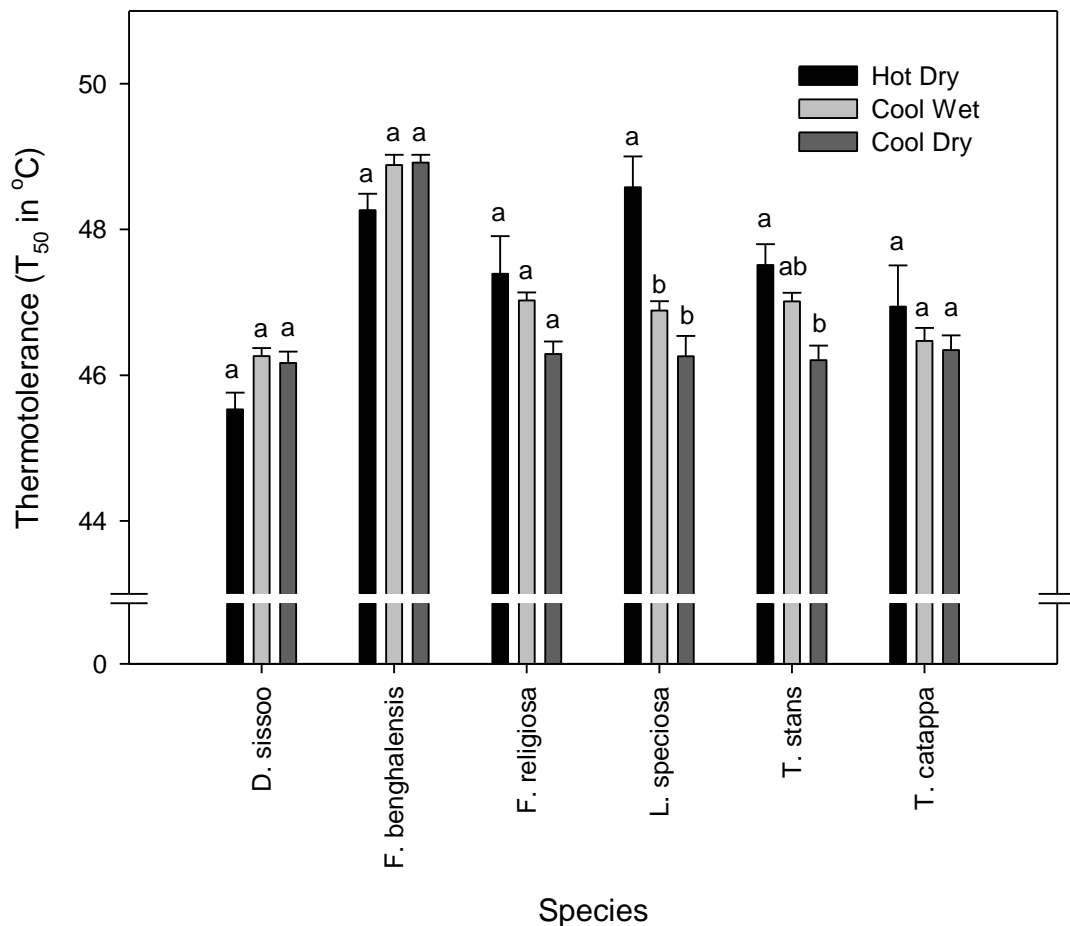


Figure 17: Variation in thermotolerance at different times of the year. The year was divided into three categories depending on the water availability and temperature conditions – hot dry (> 30°C average maximum temperature, and < 200 mm rainfall), cool-wet (< 30°C average maximum temperature, > 200 mm rainfall) and cool-dry (< 30°C average maximum temperature, < 200 mm rainfall). For more details of seasonal categories see Figure 9 and Materials and methods. The y-axis represents  $T_{50}$ , which is the temperature at which chlorophyll fluorescence drops to 50% of the controls (25°C). During each time of the year going from left to right, the bars represent *Dalbergia sissoo*, *Ficus benghalensis*, *Ficus religiosa*, *Lagetroemia speciosa*, *Tecoma stans* and *Terminalia catappa*.

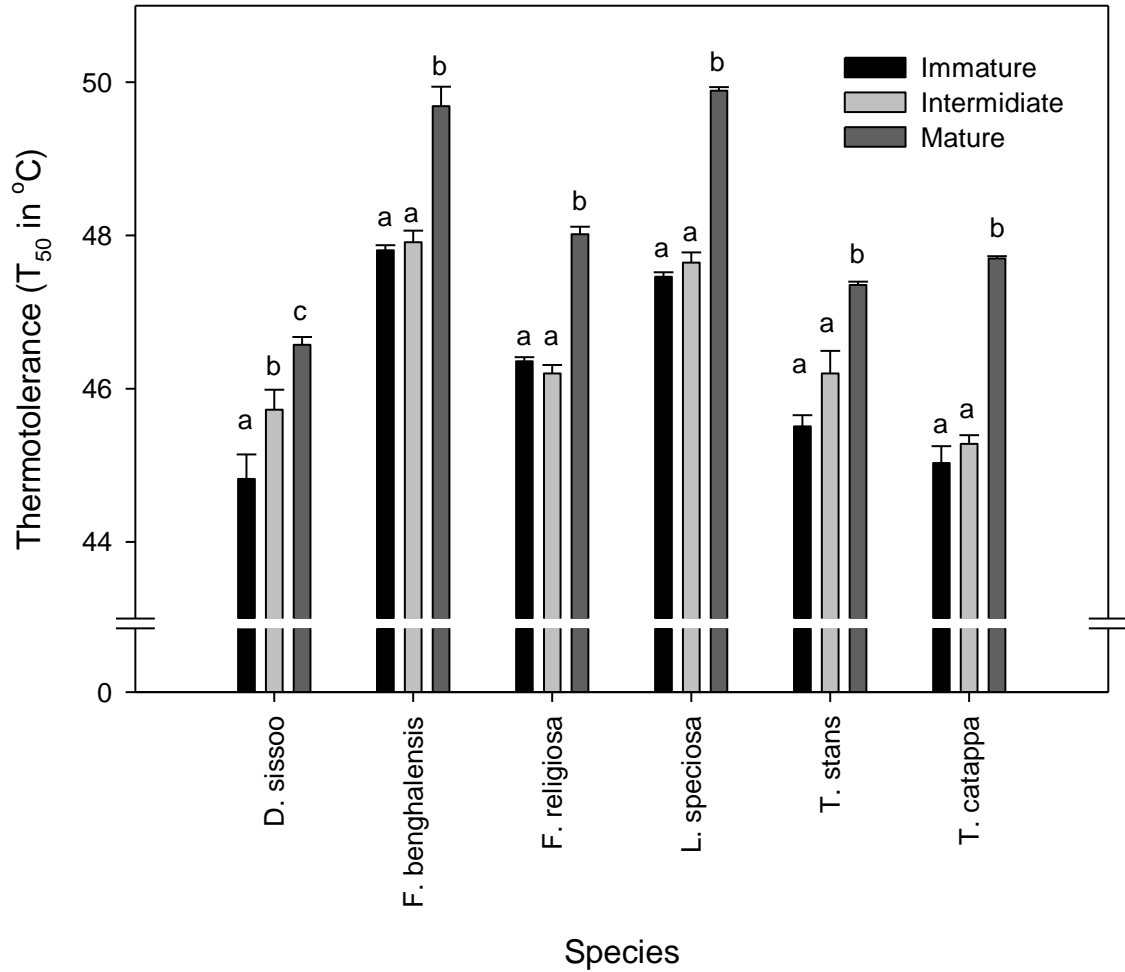


Figure 18: Developmental variation in thermotolerance ( $T_{50}$ ) between immature, intermediate and mature leaf stages. Error bars represent one standard error. Lower case letters represent homogeneous groups for comparisons within each species as assessed by Tukey's post hoc test.



## **4. Effects of drought stress on thermotolerance of tree seedlings from a tropical seasonally dry forest grown under controlled conditions**

### **4.1 Introduction**

Seasonally dry tropical forests in the Northern Western Ghats are characterized by a relatively short rainy season and a long dry season. The end of the long dry season coincides with the hottest time of the year. Thus, trees in this region have to cope with the highest temperatures in the year during a period when water is the most limiting. Future global warming scenarios predict that increased temperatures will be accompanied by more variable rainfall patterns (Niinemets 2010). This will result in an increased frequency of occurrence, and severity in extreme climatic events including heat-waves and droughts. Indeed, large scale forest die-offs in response to extreme hot and dry conditions have been documented in all continents (Allen et al. 2010). A recent study identified tropical forests, particularly in Asia and the Amazon, as extremely sensitive to climate variability and thus susceptible to future climate change (Seddon et al. 2016). However, we do not understand the consequences of extreme climatic events on tree mortality, and the effects of this on species composition (Anderegg et al. 2013). To understand how naturally occurring species deal with the combined effects of drought and high temperature stress, a greenhouse experiment was carried out with 12 species co-occurring in the northern Western Ghats.

High temperatures often occur simultaneously with low soil water availability and high vapour pressure deficit (Allen et al. 2010). Low water availability reduces the plant's ability to lower leaf surface temperatures by transpiration, and therefore heat stress is often exacerbated by water stress. In the study region, the rainy season typically starts in June and ends by the end of September with 95% of the rains in this region occur during these four months. This is followed by a long dry season, and the driest time of the year is from March to May, which is also the hottest time of the year. It is well established that growth conditions significantly affect thermotolerance in plants (Havaux 1992, Barua et al. 2008). For plants grown in controlled environments, it has been shown that drought stress confers higher thermotolerance (Havaux 1992, Ladjal et al. 2000, Gauthier et al.

2014). In fact, exposure to other abiotic stresses like salt (Lu and Zhang 1998, Lu et al. 2003) and high light (Havaux 1992) have been shown to increase thermotolerance. This phenomenon known as cross-tolerance likely results from an overlap in the mechanisms and adaptations to counter heat and drought stress. Most abiotic stresses, including drought and heat result in increased oxidative stress at the cellular level, and related damage to membranes (DarcyLameta et al. 1996), and proteins (Ristic et al. 1998).

Limitation in water availability causes stomata to shut hence reducing transpiration rates. In the absence of transpiration, leaf temperatures increase. Hence, water limitation or drought can exacerbate the heat stress that the leaf experiences. Moreover, the effects of drought and extreme temperature are similar at the cellular level and both result in increased oxidative stress and related damage to membranes, and proteins (Ristic et al. 1998). All these molecular mechanisms also confer higher thermotolerance in plants. Hence, there are common mechanisms at the molecular level to alleviate high temperature and drought stress. This phenomenon is recognized as cross-tolerance. It has also been shown that drought tolerance was positively related to temperature tolerance in 45 varieties of cereals (Havaux et al. 1988). Given that the above changes confer thermostability on PSII (Havaux 1992, Ladjal et al. 2000, Gauthier et al. 2014), one would predict that there should be a positive relationship between drought tolerance and thermotolerance of PSII.

The previous studies (Chapter 2 and Chapter 3) were conducted on individuals growing in the field in different microhabitats where access to water, exposure to light and temperatures were not controlled. The distribution of species across microhabitats is likely to be non-random and based on species-specific preferences (Lundholm and Marlin 2006). These differences could contribute to the variation in thermotolerance observed between species, and confound interpretation of the results. To address these concerns, it is necessary to grow the study individuals under controlled environmental conditions where light, water availability and temperature can be regulated. Additionally, the previous studies were conducted on trees from a semi-urban environment and included species that were not only native to this region, but also others that were naturalized or

cultivated. To address the above concerns this experimental study was designed to include species native to forests in the Northern Western Ghats. Careful attention was paid to ascertain that these species are representative of the evergreen and deciduous species found in this region, and additionally, span the range of leaf traits documented in these species.

Evergreen and deciduous are two important ecological strategies and an important plant functional trait (Wright et al. 2004, Tomlinson et al. 2014, Diaz et al. 2016). In the context of drought tolerance, evergreen trees have been known to be more stress tolerant than deciduous trees (Warren and Adams 2004, Markesteijn et al. 2011).

There has been increasing attention given to leaf functional traits like Leaf Mass per Area (LMA) as they represent important indicators of plant performance, productivity and ecological strategy, and have been shown to be a good quantitative index which allows comparisons between species (Diaz et al. 2016). High LMA species are generally slow growing, stress tolerant species, while low LMA species are fast growing and generally sensitive to stresses like drought and herbivory (Poorter et al. 2009). Leaf traits are intricately related to leaf energy budgets (Michaletz et al. 2015, Michaletz et al. 2016), and leaf temperatures (Groom et al. 2004, Vogel 2005, Leigh et al. 2012, Michaletz et al. 2016). As such it is expected that leaf traits should be related to thermotolerance (Curtis et al. 2012). However, few studies have examined the relationship between key leaf functional traits and thermotolerance, and the results from these are equivocal. Some studies have shown a positive relationship between LMA and thermotolerance (Knight and Ackerly 2003), while others show no relationship (Zhang et al. 2012) or a negative relationship (Godoy et al. 2011). The previous study (Chapter 2) found differences between evergreen and deciduous species, and a significant relationship between LMA and thermotolerance in field grown individuals of forty-one species examined. The present study examines the variation in thermotolerance and the effect of drought stress on tree saplings when grown under controlled conditions.

Given the threat of concomitant occurrence of drought and high temperature stress, and the lack of information of how naturally growing tropical trees would respond to such integrated stressful events, the following questions were asked in saplings of native trees:

- a) Are evergreen species more thermotolerant than deciduous species?
- b) Are leaf traits related to thermotolerance?
- c) Does drought stress affect thermotolerance?
- d) Do species with high thermotolerance have higher drought tolerance?

## **4.2 Materials and Methods**

### **Species selection and growth conditions**

We selected 12 trees that are commonly found in the seasonally dry forests of the Northern Western Ghats of peninsular India. The vegetation in this region varies from scrub/savanna to semi-evergreen forests. The climate is seasonal and most of the annual rainfall of 2266mm falls between June and September. Monthly minimum temperatures in January average 11°C while maximum temperatures in April average 35°C. Ten of the 12 species were selected from 80 species for which leaf functional trait, and ~3 years of leaf phenology were available (D. Barua unpubl. data). This allowed us to identify and select representative species that span the range of leafing behaviour and leaf functional trait values observed in this region.

The study was conducted between May and July 2015 at the Indian Institute of Science Education and Research (IISER) campus, Pune, India. For all species, 2.5-year-old saplings were obtained from a local nursery (J.E. Farms, Pune), and transplanted to 19 L PVC pots (60 cm length, and 20 cm diameter) filled with 18 kg dry red alfisol (pH 7.2) supplemented with organic manure (1:50 v/v). Saplings were moved to a greenhouse and given a period of 6 weeks to acclimate before the experiment. All plants were fertilized once, 15 days after transplantation, with urea (0.05 g·kg<sup>-1</sup> soil). The greenhouse received natural sunlight supplemented with incandescent lamps to ensure photosynthetic photon flux density (PPFD) of 500-800 μmol·m<sup>-2</sup>·s<sup>-1</sup> (between 09:00 and 16:00 h). Mean daily temperature in the greenhouse ranged between 25 and 31°C, while relative humidity (30-55%) varied according to local conditions.

### **Drought treatments**

At the start of the experiment, 12 individuals of each species were randomly assigned to the two treatments (6 control - well watered; 6 drought) and randomly allocated positions in the greenhouse. Before the beginning of the treatments, all pots were fully saturated with water in the evening, excess water allowed to drain overnight, and weighed the next morning to determine the pot weight at field capacity. A white plastic sheet was taped to the pot rim and loosely tied around the base of the plants to minimize evaporation from

the soil. During the experiment all control pots were individually weighed every 3 days, the loss of water quantified, and the appropriate volume of water added to bring the pot back to 90% of its field capacity. In this manner all control plants were always maintained at 75-90% field capacity.

Drought was imposed by termination of watering at the start of the experiment. These pots were weighed every 3 days to estimate water loss and determine the point at which the pot water reached 30% of field capacity. To standardize the drought treatment across these species that varied widely in their water use, we used the time at which pots reached 30% of field capacity as the end point of the drought treatment. The effect of drought was not examined for *Garcinia indica* because of the lack of sufficient plants. At the end of the drought treatment we measured gas exchange, quantified leaf wilting, and collected leaf samples for thermotolerance assays and estimation of leaf relative water content.

#### **Estimation of leaf wilting stage and relative water content (RWC)**

Average leaf wilting stage scores were estimated and RWC quantified in the morning after drought stressed plants reached 30% of field capacity. Average leaf wilting was scored in a semi-quantitative manner (Engelbrecht and Kursar 2003) for the 3rd, 4th and 5th leaves from the apex for 5-6 individuals per species. Wilting was assessed as change in leaf angle relative to the stem axis as compared to control leaves, by rolling and folding of leaves, and necrosis and chlorosis, and scored from 1-5 based on categories defined by Engelbrecht et al. (2003). Briefly, stage 1 - no signs of wilting or damage; stage 2 - slight change in leaf angle, but no rolling or folding; stage 3 - pronounced change in leaf angle or protrusion of veins; stage 4 - extreme change in leaf angle with beginning of cell death; 5 - complete necrosis of the leaf.

For quantification of leaf RWC, leaf discs (1cm<sup>2</sup>) were excised with a cork-borer from the middle of the first fully expanded mature leaf taking care to exclude the mid-vein. The discs were weighed to quantify fresh weight (FW), water saturated for 24 hr at 4°C, and the turgid fresh weight (TW) measured. Discs were then put in a drying oven at 70°C

for 3-4 days till a constant dry weight was obtained. Leaf relative water content (Saura-Mas and Lloret 2007) was calculated as:  $RWC = 100 \times (FW-DW)/(TW-DW)$  for 6 individuals of each species.

### **Gas exchange measurements**

Leaf gas exchange was measured for the first fully expanded leaf for 6 individuals each for control (well-watered) and drought stressed plants (at the end of the drought treatment) with a LI-6400XT Portable Photosynthesis System (LI-COR, Lincoln, USA) using the standard broad-leaf cuvette (6 cm<sup>2</sup>) fitted with the LICOR-6400-02B LED light source. These measurements were made between 9:30-11:30 h with light, chamber CO<sub>2</sub> concentrations, relative humidity and temperature set at 800  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  PPFD, 390 + 10 ppm, 50-60%, and 28-30°C, respectively. All gas exchange measurements were made after the leaf temperature equilibrated with the cuvette temperature.

### **Quantification of leaf functional traits**

Leaf traits - leaf mass per area (LMA, g<sup>1</sup>m<sup>-2</sup>), leaf dry matter content (LDMC, mg<sup>1</sup>g<sup>-1</sup>), and leaf area (LA, cm<sup>2</sup>) were quantified for 5 fully expanded and mature leaves from 6 individuals of the control (well-watered) plants as per protocols recommended (Pérez-Harguindeguy et al. 2013). Leaves were water saturated for 12 hr at 4°C, and the saturated fresh weight obtained. They were then scanned with a desktop scanner to quantify leaf area, and put in a drying oven at 70°C for 3-4 days till a constant dry weight was obtained. LMA was quantified as the ratio of dry weight to one sided leaf surface area, and LDMC as the ratio of dry weight and saturated fresh weight.

### **Temperature tolerance assays**

For control (well-watered) plants, we measured the temperature response of dark adapted chlorophyll a fluorescence ( $F_v/F_m$ ), and estimated the temperature that results in the 50% loss of function ( $T_{50}$ ).  $F_v/F_m$  represents the maximum potential quantum yield of photosystem II (PSII) and was calculated as  $F_v/F_m = (F_m - F_o)/ F_m$ , where  $F_m$  and  $F_o$  are the maximum and basal fluorescence yield, respectively, for dark adapted leaves. Leaf discs (2 cm<sup>2</sup>) were placed between two layers of muslin cloth, covered with

aluminium foil and put in a sealed zip lock bag with moist tissue at the bottom to keep the bag water saturated. This was immersed in a temperature controlled refrigerated water bath (Julabo, Model F25, Seelbach, Germany) set to the desired temperature (25°C, 40°C, 45°C, 47.5°C, 50°C) for 30 min. Preliminary trials and previous studies (Curtis et al. 2014) showed that a 30-min exposure resulted in irreversible damage and negligible recovery. The temperature of dummy leaf discs (not used for assays) was monitored with a thermocouple attached to the underside of the disc. Preliminary trials determined the temperature of the water bath required to maintain the desired leaf temperatures. Following temperature treatment, leaf discs were allowed to dark adapt for 30 min in the dark in a water saturated environment at room temperature before measurement of  $F_v/F_m$  using a PAM 2500 fluorometer (Walz, Effeltrich, Germany).

A four-parameter logistic sigmoid curve was fitted to the chlorophyll a fluorescence ( $F_v/F_m$ ) values across the range of temperatures examined using the R package 'drc' (Ritz and Streibig 2005). The parameters included in the model are the upper asymptote, the lower asymptote, the steepness of the curve and the point on the X-axis at which the value on the Y-axis reduces to half of the upper asymptote. The four-parameter model with the lower asymptote set to zero was observed to generate appropriate curves. The temperature ( $T_{50}$ ) at which reduction in  $F_v/F_m$  was 50% of the upper asymptote was estimated from these curves. We used 5 independent leaf discs from an individual at each of the temperatures to generate an  $F_v/F_m$  response curve from which we estimated  $T_{50}$  for that individual. This was repeated for 5- 6 replicates individuals for each species. For the drought stressed plants we measured  $F_v/F_m$  at 25°C, 47.5°C, 50°C due to limited availability of leaf samples, and thus did not calculate  $T_{50}$ .

### **Statistical analyses**

We examined variation in  $T_{50}$  of PSII function for the control plants using a mixed model ANOVA with leaf habit (evergreen and deciduous) as a fixed effect, and species as a random effect nested within leaf habit. To test how experimentally imposed drought affects thermotolerance in these species we examined variation in  $F_v/F_m$  using an ANOVA with species, treatment (control and drought) and temperature (25°C, 47.5°C,



50°C) as fixed effects. Variation in LMA, LDMC, LA, wilting score, and RWC were examined using ANOVA with species as a fixed effect. We performed non-parametric Kruskal Wallis tests for LMA, LA and wilting scores as these were not normally distributed. Variation in photosynthetic rates was examined with a 2-way ANOVA with species and treatment (control and drought) as fixed effects.

We used Pearson's correlations to examine relationships between thermotolerance ( $T_{50}$  of PSII for control, and  $F_v/F_m$  at 47.5°C for drought treatments), leaf traits (LMA, LDMC, LA and photosynthetic rates), and drought tolerance (leaf RWC, wilting score, change in photosynthetic rates with drought). Additionally, we examined Spearman's rank correlations for analyses with LMA, leaf area and wilting scores as these variables were not normally distributed. All analyses were performed using Statistica (version 9.1, Statsoft, Tulsa, OK, USA).

### 4.3 Results

Temperature response curves of efficiency of PSII function were similar in shape for the different species. There was no change in the efficiency of PSII at 40°C but the PSII efficiency reduced at higher temperatures. For some sensitive species PSII function was reduced to zero at 47.5°C, while others it was reduced to zero at 50°C. *Olea dioica* did not hit zero even at 52.5°C. Thermotolerance (temperature at which dark-adapted chlorophyll fluorescence was 50% of controls – T<sub>50</sub>) was higher for evergreen species (46.9°C) than deciduous species (45.5°C; Table 9 and Figure 20). Species had differences in thermotolerance as well (Table 9 and Figure 20) with means ranging from 44.44°C to 48.09°C.

To examine the effect of drought stress on thermotolerance, plants were either well-watered or drought stressed as described in the methods and materials. As the number of leaves available for assay was limited in the drought stressed plants heat treatment was only performed at three temperatures only – 25°C, 47.5°C and 50°C. Hence, the estimation of T<sub>50</sub> from the temperature response curve was not possible in this experiment. At 25°C, there was no discernible difference in dark-adapted chlorophyll fluorescence of the well-watered and drought stressed plants, while dark-adapted chlorophyll fluorescence was higher for drought stressed plants than well-watered plants at 47.5°C and 50°C (Table 9 and Figure 28). Variation in the effect of drought on thermotolerance was the highest at 47.5°C and therefore for further analyses of the effect of drought on thermotolerance, dark-adapted chlorophyll fluorescence at 47.5°C was used as an estimate of thermotolerance. From this analysis, it was evident that drought treated plants had higher thermotolerance than control plants (Table 9 and Figure 21). Leaf habit had no discernible effect on thermotolerance (Table 9 and Figure 21). Thermotolerance of different species was different. All species, evergreen and deciduous showed higher thermotolerance in drought treated plants than well-watered plants (Table 9 and Figure 21).

In this experiment, three measures were used – relative water content (RWC), wilting stage (WS) and difference in net photosynthesis between well-watered and drought

stressed plants ( $\Delta P_{\text{net}}$ ) – were used to assess drought tolerance. Higher RWC meant higher drought tolerance, lower wilting stage indicated higher drought tolerance and low  $\Delta P_{\text{net}}$  indicated higher drought tolerance. Thermotolerance of well-watered plants was positively correlated with relative water content and negatively correlated with  $\Delta P_{\text{net}}$ . The negative correlation between thermotolerance of well-watered plants and wilting stage was moderately significant (Table 10). Similarly, thermotolerance of drought stressed plants (dark-adapted chlorophyll fluorescence at 47.5°C) was positively correlated with relative water content, negatively correlated with  $\Delta P_{\text{net}}$ . Thermotolerance showed moderate negative correlation with wilting stage. These results indicate that species with thermotolerance had high drought tolerance as well.

Thermotolerance of well-watered and drought stressed plants were positively correlated with leaf mass per area. However, no significant relation was found between either of the thermotolerance measures and leaf dry matter content and leaf area (Table 10).

While not a primary objective for this study, it was seen that only LMA was found to be negatively related to  $\Delta P_{\text{net}}$  (Table 10).

The thermotolerance estimates, the drought tolerance estimates and leaf traits were not normally distributed and so the non-parametric Kruskal-Wallis tests were performed, which had qualitatively the same results as the above ANOVA. Spearman's rank correlation was carried out for all the parameters for which correlations were performed. The rank correlations were qualitatively similar to the Pearson's correlations described earlier.

#### 4.4 Discussion

In this study, it was shown that drought stress increases thermotolerance. Additionally, species with high thermotolerance also had high drought tolerance. As seen in the previous study on field based trees, thermotolerance was higher for evergreen species than deciduous species and thermotolerance was related to leaf mass per area (LMA). Given the climate change related increases in temperatures and frequency of droughts, these results could have important implications in view of global warming and could lead to directional die-offs in communities leading altered species composition, community structure and ecosystem function.

The range of thermotolerance for the twelve-species grown under controlled conditions was similar to range observed for the 41 species in the field study (Chapter 2). However, mean thermotolerance in these twelve species was lower than in the previous study. This could be due to the conditions in the greenhouse, where light levels were lower and temperature was controlled between 25°C and 28°C, which was lower than the temperatures the field tree species experience. Additionally, this study was conducted with three-year old saplings that were well-watered. As seen in the previous study, evergreen species had higher thermotolerance than deciduous species, and thermotolerance was positively related to LMA and no relationship with leaf area. This supports evidence from the field data, and supports the claim that these patterns in the field species were not due to differences in growing conditions, age of the tree or microhabitats.

Importantly, it was seen that thermotolerance was higher when plants were drought stressed. This has been reported for crops (Havaux 1992, Epron 1997, Valladares and Pearcy 1997, Lu and Zhang 1999) but has been shown for naturally occurring trees in very few studies (Havaux 1992, Epron 1997, Valladares and Pearcy 1997, Lu and Zhang 1999, Ladjal et al. 2000). This complements the observation that in tropical dry deciduous forests, tree species have higher thermotolerance during the hot-dry season than the cool-wet season. Higher thermotolerance in drought stressed plants likely contributes seasonal variation in thermotolerance in these species.

There was a positive correlation between thermotolerance and drought tolerance, but the direction of causality is very difficult ascertain from the data presented. However, from studies on crops and model organisms, it is known that there are common molecular mechanisms which help the cell to cope with high temperature stress and drought stress (Vierling and Nguyen 1992, DarcyLameta et al. 1996, Ristic et al. 1998, Krause et al. 2006). Some examples of these are proteins, like super oxide dismutase (SOD), which are involved in maintaining the reactive oxygen species to manageable levels or the induction of the zeaxanthin cycle (Krause et al. 2006). There are some proteins like heat shock proteins (HSPs) are chaperones and help keeping the functional conformation of other proteins (Vierling and Nguyen 1992).

One needs to be cautious about extrapolating patterns from results obtained from twelve species only. However, the phenology and LMA of these species was known. The selected species spanned from highly deciduous to semi-deciduous to evergreen. Similarly, the range of LMA spanned the range of LMA observed for woody species from this region.

Overall, the results suggest that deciduous species and those with lower LMA may be more vulnerable to heat and drought stress, and will be more severely affected by future climate change. This would lead to directional compositional shift towards evergreen species with higher LMA in light of future climates with increased frequency of extreme climatic events - heat waves and droughts. Directional compositional changes could alter feedbacks to the atmosphere via carbon and water cycles. Evergreen, high LMA species are more conservative in water use and slow down nutrient as evergreen and high LMA species are more recalcitrant. Altered carbon cycles and atmospheric feedback has the potential to alter the rates of future climate change. Increased abundance of slow growing and less productive evergreen species with higher LMA could decrease sink strength and exacerbate future global warming.

#### 4.5 Tables and figures

Table 8: List of species used for the study. All species used are native to the northern Western Ghats.

<b>Code</b>	<b>Species</b>	<b>Leaf habit</b>
BR	<i>Bridelia retusa</i> (L.) A.Juss	Deciduous
DM	<i>Diospyros montana</i> Roxb	Deciduous
GI	<i>Garcinia indica</i> (Thouars) Choisy	Evergreen
HF	<i>Heterophragma quadriloculare</i> (Roxb.) K.Schum.	Deciduous
ME	<i>Mimusops elengi</i> L.	Evergreen
MI	<i>Mangifera indica</i> L.	Evergreen
MU	<i>Memecylon umbellatum</i> Burm. f.	Evergreen
OD	<i>Olea dioica</i> Roxb.	Evergreen
SC	<i>Syzygium cumini</i> (L.) Skeels	Evergreen
SO	<i>Schleichera oleosa</i> (Lour.) Merr.	Deciduous
TB	<i>Terminalia bellirica</i> (Gaertn.) Roxb.	Deciduous
TC	<i>Terminalia chebula</i> Retz.	Deciduous

Table 9: Variation in thermotolerance in species and with different water availability a) Variation in thermotolerance ( $T_{50}$  of PSII function) between leaf habit (evergreen and deciduous) and species. Results shown are for a mixed model ANOVA with species nested within leaf habit. b) Variation in thermotolerance ( $F_v/F_m$  - PSII function at 47.5°C) for 11 species under control (well-watered) and drought stressed conditions.

<b>Effect</b>	<b>df</b>	<b>MS</b>	<b>F</b>	<b>p</b>
<b>a) Thermotolerance - <math>T_{50}</math> of PSII function (control plants)</b>				
Leaf Habit	1	29.7	6.09	< 0.05
Species (leaf habit)	10	4.9	5.25	< 0.001
<b>b) PSII function at 47.5°C (control vs. drought)</b>				
Drought	1	1.26	74.13	<0.001
Species	10	0.1	5.88	<0.001
Drought x Species	10	0.009	0.51	0.879

Table 10: Relationship of thermotolerance with leaf traits and drought tolerance.

Relationship between thermotolerance ( $T_{50}$  for PSII function - control plants, and  $F_v/F_m$  - PSII function at 47.5°C for drought stressed plants) and: a) Leaf traits - leaf mass per unit area (LMA), leaf dry matter content (LDMC), and leaf area (LA); b) Net photosynthesis for control plants ( $P_n$ -C), and after drought stress ( $P_n$ -D); c) performance under drought stress - decrease in photosynthesis ( $\text{del-}P_n$ ), leaf wilting stage scores (WS), leaf relative water content (RWC). Values represent Pearson's correlation coefficients and those in bold are significant at  $p < 0.05$ . For all analyses with  $T_{50}$  (control plants)  $n=12$ , and for those with  $F_v/F_m$  - at 47.5°C (drought stressed plants)  $n=11$ .

		<b><math>T_{50}</math> - control</b>	<b><math>F_v/F_m</math> @ 47.5°C</b>
		<b>Well-watered</b>	<b>drought</b>
<b>a) Leaf traits</b>	LMA	<b>0.6715, p=0.024</b>	<b>0.8327, p=0.001</b>
	LDMC	0.3066, p=0.359	0.4563, p=0.158
	LA	-0.3321, p=0.318	-0.3116, p=0.351
<b>b) Photosynthesis</b>	$P_n$ -C	<b>-0.7283, p=0.011</b>	<b>-0.7415, p=0.009</b>
	$P_n$ -D	0.4925, p=0.124	0.3497, p=0.292
<b>c) Drought tolerance</b>	$\text{del-}P_n$	<b>-0.7616, p=0.006</b>	<b>-0.7229, p=0.012</b>
	WS	-0.5345, p=0.090	-0.5942, p=0.054
	RWC	<b>0.7342, p=0.010</b>	<b>0.6366, p=0.035</b>



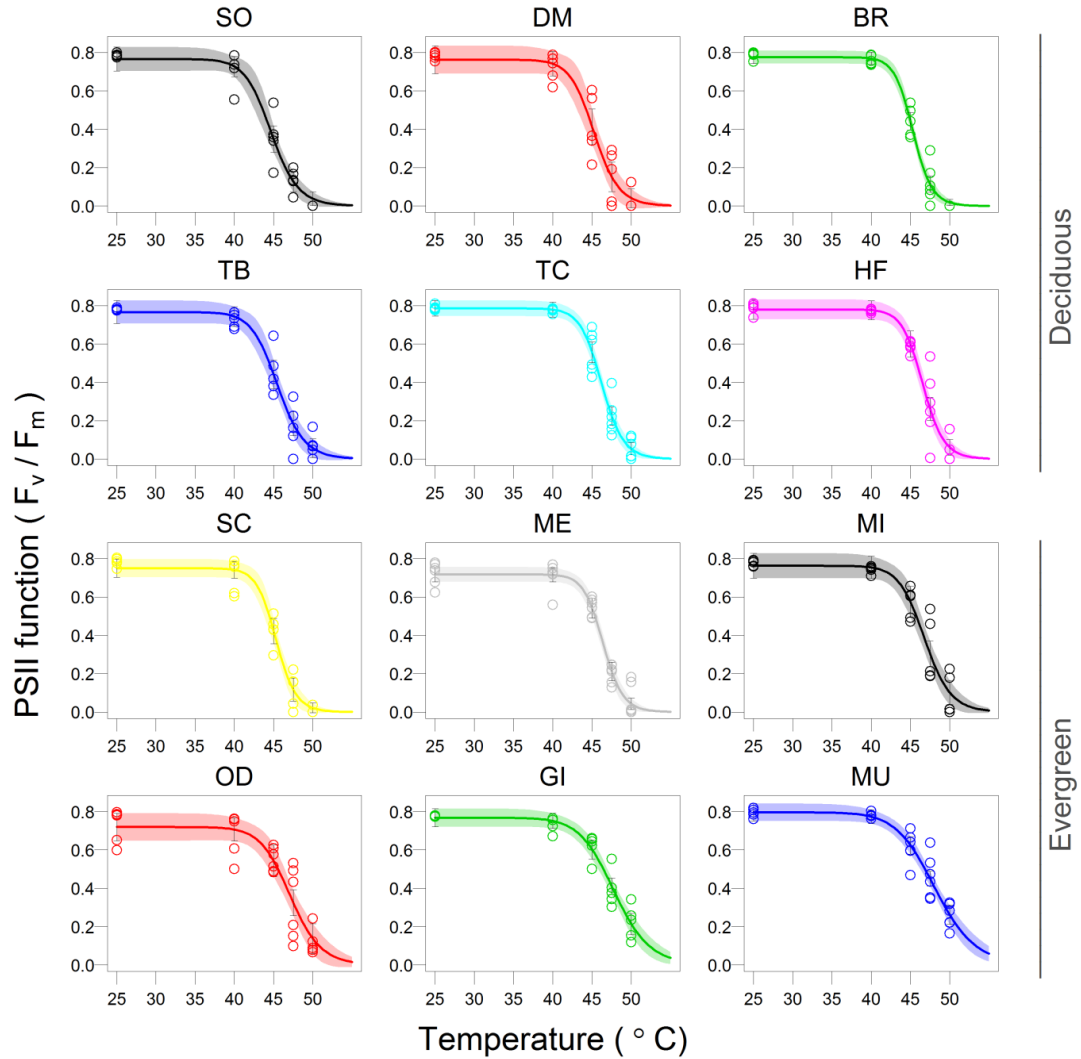


Figure 19: Temperature response of photosystem II function (dark adapted chlorophyll *a* fluorescence -  $F_v/F_m$ ) in control (well-watered) plants of the 12 tropical trees examined. The open circles represent the  $F_v/F_m$  values, the line represents a logistic sigmoid fit, the errors bars and the shaded portion indicates the 95% CI ( $n = 5 - 6$  individuals for each species). Species are arranged in increasing order of thermotolerance for deciduous and then evergreen leaf habits. We follow this same sequence in subsequent figures for ease of comparison across figures. Species names provided in Table 8.

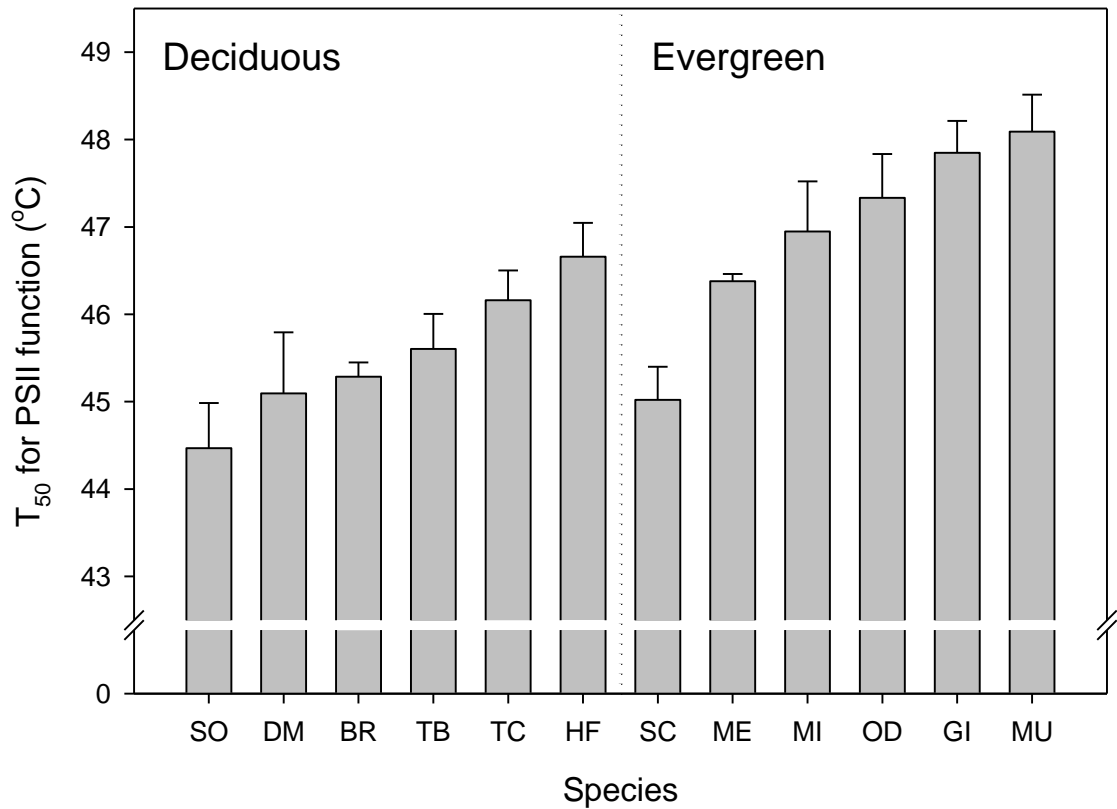


Figure 20: Thermotolerance ( $T_{50}$  - temperature for 50% of reduction in PSII function as measured by dark adapted  $F_v/F_m$ ) in control (well watered) plants of the 12 study species. Estimates for  $T_{50}$  were calculated from the sigmoid logistic curves fitted for 5-6 replicate individuals for each species. Error bars represent standard error ( $n = 5 - 6$ ). Species names are provided in Table 8.

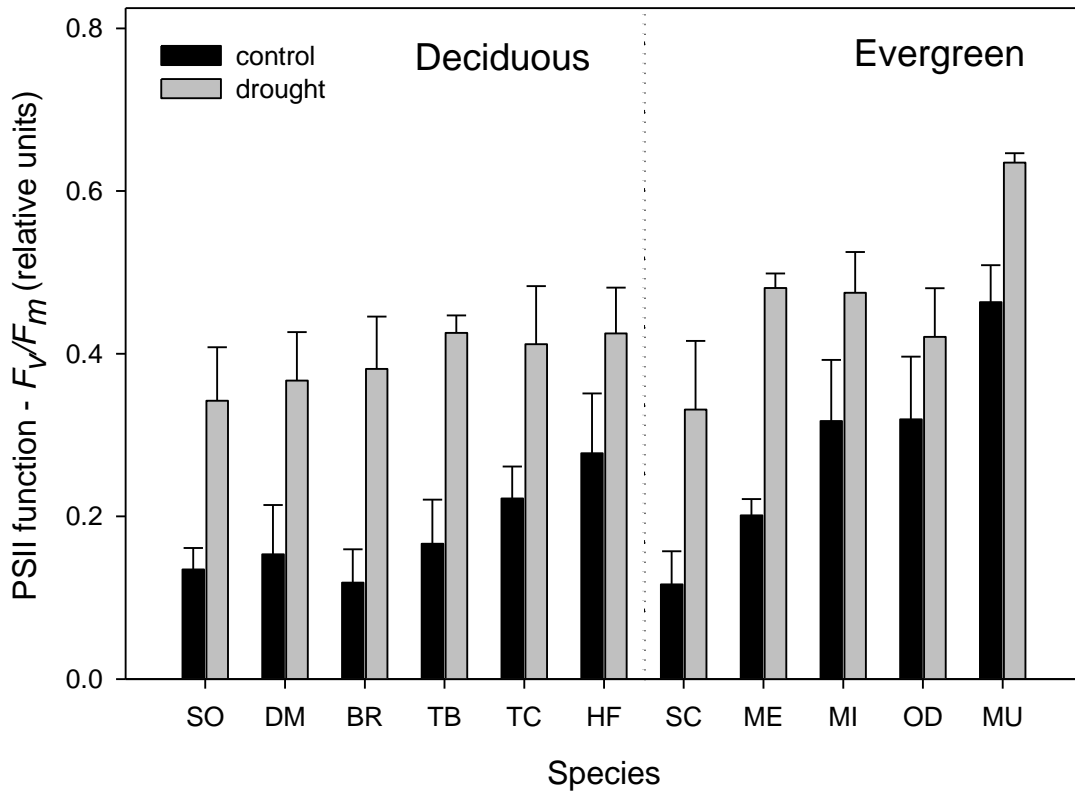


Figure 21: The effect of drought stress on dark adapted chlorophyll *a* fluorescence ( $F_v/F_m$ ) at 47.5°C. Dark and grey bars represent control (well-watered) and drought stressed plants, respectively. Error bars represent standard error (n = 5 - 6). Species names are provided in Table 8.

## 5. Conclusion

The results of the study of forty-one trees from a seasonally dry tropical region showed that there was significant variation in high temperature tolerance measured as the temperature at which there is a 50% reduction in Photosystem II function ( $T_{50}$ ). This variation ranged from 45°C in the most sensitive species to 51°C in the most tolerant one. Broad-leaved evergreen trees had higher thermotolerance than dry deciduous species. The relationship between deciduousness and thermotolerance was congruent with the qualitative differences in thermotolerance between evergreen and deciduous trees; there was a significant negative relationship between deciduousness and thermotolerance. Additionally, it was seen that thermotolerance was positively related to the key leaf functional trait, leaf mass per area (LMA). Thus, evergreen species, which also have thicker leaves with higher LMA, are relatively more thermotolerant than deciduous trees. Results from these studies also show that there is significant seasonal variation within species in thermotolerance between the hot-dry and the cool-wet rainy season. These results were corroborated in the subsequent study that examined annual variation in tolerance in a subset of six species. Here a significant leaf developmental stage related variation in tolerance was found suggesting that recently flushed, young immature leaves are more sensitive to heat stress, and likely to be more severely affected on exposure to extreme temperatures. The final study examined the importance of water availability on thermotolerance, and corroborated results obtained from the field study. Here saplings from twelve species were grown under common controlled conditions, and as before it was seen that evergreen trees had higher tolerance, and that tolerance was positively related to the key leaf functional trait, LMA. Additionally, drought stressed plants had higher thermotolerance, and results suggest that tolerance to drought is positively related to tolerance to extreme temperatures.

Thermotolerance from the present study (mean = 47°C) was comparable to thermotolerance of tropical species obtained from literature (mean = 46°C).

Thermotolerance from the present study was comparable to thermotolerance for species from the temperate regions (mean = 46°C). Interestingly, mean thermotolerance of woody species from the tropics and temperate regions were identical – 46.4°C for tropical woody

species, and 46.6°C for temperate woody species. Thermotolerance for tropical woody species in literature ranged from 34°C to 57°C. The range of thermotolerance from the present study was much lower going from 45°C to 49°C. The range of thermotolerance of the present study was not only lower than those for sites in the temperate regions (Gauslaa 1984, O'Sullivan et al. 2017), but also lower than most sites which had studied more than ten species per site (Weng and Lai 2005, O'Sullivan et al. 2017).

A recent study (O'Sullivan et al. 2017), estimated thermotolerance from 18 sites spanning a wide range of latitudes. They measured respiratory rates and chlorophyll fluorescence as measures of thermotolerance in woody species. For the discussion here, only results from the chlorophyll fluorescence are referred to, as the critical temperatures of respiration are too high and may not be ecologically relevant. They used the dynamic method of assessing chlorophyll fluorescence, and they estimated critical temperatures ( $T_c$ ) for their study species. They showed that sites with higher maximum habitat temperature had higher thermotolerance ( $T_c$ ). However, the relationship between thermotolerance and habitat temperature was shallow – for about a 30°C difference in habitat temperature there was an 8°C difference in thermotolerance. However, one needs to be cautious in drawing general conclusions from this study (O'Sullivan et al. 2017) as half of the sites in the study are from Australia (nine) – four in North America, one from Europe and there are only four sites from the Neo-tropics, out of which one is a high-altitude site.

In order to get a comprehensive understanding of the relationship between thermotolerance and habitat temperature, a comprehensive literature review and meta-analysis was conducted for studies that have examined thermotolerance in woody species. To ensure that data could be combined and compared this was restricted to studies that used chlorophyll fluorescence. This included a total of 32 studies covering 50 sites and about 300 species. Even within these studies, there were two broad differences in the methodology used; dynamic assays entail exposing leaves to steadily increasing temperature, while static assays expose leaves to a set temperature for a fixed duration of time. Therefore, two separate analyses were conducted, the first of which used static

assays, with thirty-minute exposure to the treatment temperature and the temperature at which dark-adapted chlorophyll fluorescence is 50% of controls ( $T_{50}$ ) is estimated. This is directly comparable to the methodology used in the thesis. The second analysis included studies that included dynamic assays and a common  $1^{\circ}\text{C}/\text{min}$  treatment regime which generates the temperature at which rapid rise in chlorophyll fluorescence occurs – called critical temperature ( $T_c$ ).

The first analysis which used exactly the same method as used in the present study, included 11 studies covering around 29 species. There were a significantly greater number of studies with a much larger coverage of species that used the dynamic method of estimating thermotolerance (21 studies, around 284 species over 38 sites). To better understand the relevance of estimates of  $T_{50}$  from the present study, the  $T_{50}$  values were converted equivalent  $T_c$  values (as done by Araujo 2013). For the studies which report both – dynamic ( $T_c$ ) and static ( $T_{50}$ ) estimates of thermotolerance, the relationship between  $T_c$  and  $T_{50}$  was examined (Figure 29, 17 species). Maximum temperature of the hottest month was derived from a high resolution global dataset (Mark et al. 2002) and was used as an index of maximum temperature of the habitat. The relationship between thermotolerance and habitat temperature was examined.

For the static assay ( $T_{50}$ ) comparison, there was a significant positive relation between thermotolerance and the maximum temperature of the hottest month, but this relationship was shallow – a  $6^{\circ}\text{C}$  difference in thermotolerance for a  $17^{\circ}\text{C}$  difference in maximum habitat temperature (Figure 22). Thermotolerance of species from the current study was lower than what would be expected for species from a habitat with such high temperatures. However, this analysis was limited by the number of species for which data was available.

As seen in the previous analysis of the  $T_{50}$  analysis, the relationship between thermotolerance ( $T_c$ ) and maximum habitat temperature of the habitat was examined. Here too, there was a significant positive relation between thermotolerance and maximum temperature of the hottest month – a  $10^{\circ}\text{C}$  difference in thermotolerance for a  $35^{\circ}\text{C}$

difference in habitat temperatures (Figure 23). Similar to the previous analysis, the mean thermotolerance of species from the present study was lower than what would be globally expected for habitats with such temperatures. Additionally, the maximum thermotolerance of species from the present study was much lower than what would be expected, while minimum temperatures did not seem different than other sites with similar maximum habitat temperatures. The range of thermotolerance for fifty-three species examined from the present study was much lower than other sites. The studies with similar or lower number of species examined had greater ranges of thermotolerance.

Although the shallow relationship between thermotolerance and habitat temperature has been previously recognized (Gauslaa 1984, Araujo et al. 2013, O'Sullivan et al. 2017), the reason for this is not well understood. One explanation for this is that thermotolerance of plants is much higher than the highest temperatures that they experience. Thus, this variation in thermotolerance may have little adaptive significance. Such a pattern could be a relic of high temperatures experienced through their evolutionary history (Dick et al. 2013). Alternatively, the shallow relationship between thermotolerance and habitat temperature could be a result of the fact that air temperatures that are used as a measure of habitat temperature, do not reflect actual leaf temperatures experienced. Given enough water, leaf temperatures can be significantly lower than air temperatures (Michaletz et al. 2015, Michaletz et al. 2016). However, when water availability is low and transpirational cooling is compromised, leaf temperatures for leaves in the open sun can be 5°C-20°C higher than air temperatures (Vogel 2005, Leigh et al. 2012).

To better understand what the functional consequences of the observed estimates of thermotolerance are for these plants, it was necessary to understand what temperatures they experience in their natural habitats. Additionally, to use these estimates of thermotolerance to predict how these species may be affected by global warming it is important to understand the range of potential temperatures they may experience in future climates. Average temperature in the tropics are predicted to rise by 3-6°C by the end of the century (Malhi and Wright 2004, Malhi et al. 2014). The maximum temperature experienced in the study region during the last ten years is 42.05°C. In the current

climate, the difference between the  $T_{50}$  of the most sensitive species ( $45^{\circ}\text{C}$ ) and the maximum temperature (thermal safety limit) is about  $3^{\circ}\text{C}$ . A  $3^{\circ}\text{C}$  increase in maximum temperatures could mean that the most sensitive species will have no or a very narrow thermal safety margin. A  $6^{\circ}\text{C}$  increase in maximum temperatures would lead to about 50% of the species having  $T_{50}$  lower than the temperatures experienced (Figure 25). However, this is a conservative estimate of functional effects of thermotolerance on species from the current study. This is assuming that leaf temperature is equal to air temperature. During conditions of limited water availability, leaf temperatures can be  $5^{\circ}\text{C}$ - $20^{\circ}\text{C}$  higher than air temperatures (Groom et al. 2004, Vogel 2005, Leigh et al. 2012).

If leaf temperature is same as the air temperature, in the current climate none of the species will have leaf temperature higher than their  $T_{50}$  and no day in the year will have temperatures hotter than the mean  $T_{50}$  of all the species. If leaf temperatures are  $5^{\circ}\text{C}$  higher than the air temperature, then leaf temperature of 32% of the studied species can go higher than their respective  $T_{50}$ s and this number goes up to 100% if leaf temperature is  $10^{\circ}\text{C}$  higher than air temperature (Table 11). In case of a  $6^{\circ}\text{C}$  increase in maximum temperature, all the species will have leaf temperatures higher than their respective  $T_{50}$ s. However, the number of days for which the effect will remain will be different depending how much higher leaf temperature is than air temperature (Table 11). This reiterates the need to understand temperatures experienced by the leaves in their natural habitats.

In the seasonally dry regions of the northern Western Ghats, the hottest and driest time of the year coincide and plants have to deal with multiple stresses simultaneously. Given that water availability and hence transpirational cooling is limited during this time, and high light leaf, temperatures are likely to be significantly higher than air temperatures. Additionally, the majority of the species in this region flush leaves during the hot-dry season and the present study has shown that developing leaves are more sensitive to high temperature stress than mature leaves. Taken together, it suggests that trees from seasonally dry tropical forests may be particularly vulnerable to future climate change related global warming.



Results from this study show that differential effects between species and importantly these differential effects are not random with respect to plant functional type and leaf mass per area (LMA). Climate change related global warming could lead to directional changes in species composition in seasonally dry tropical forests, favouring slow growing evergreen species with high LMA. This would lead to altered ecosystem function, including decreased productivity and nutrient cycling due to increased proportion of slow growing, high LMA species. Species with leaves that have high LMA have lower rates of carbon assimilation and this could alter soil-atmosphere feedbacks and this is important fundamentally in understanding and predicting not just plant responses to future climate change but also the rate and magnitude of the change.

## 5.1 Tables and figures

Table 11: For current and future climates: a) The percentage of species that will experience leaf temperature greater than their  $T_{50}$ ; and, b) The range of days in a year when leaf temperatures ( $T_{\text{leaf}}$ ) exceed the  $T_{50}$ . We consider three estimates of leaf temperature: i) Leaf temperatures = air temperature; ii) Leaf temperatures = air temperature + 5°C; iii) Leaf temperatures = air temperature + 10°C. Daily air temperature data for 10 years (2006-2015) were obtained from GHCN (Global Historical Climatology Network) daily Version 3.22. Future air temperature estimates are the upper and lower limits of the predicted increases of 3-6°C in mean temperatures for tropical regions by the year 2100 (Malhi and Wright 2004, Malhi et al. 2014).

Estimated $T_{\text{leaf}}$	Current climate	Future climate	
		+3°C	+6°C
<b>a) Percentage of species examined that will experience <math>T_{\text{leaf}} &gt; T_{50}</math></b>			
i) $T_{\text{leaf}} = T_{\text{air}}$	0	0	61
ii) $T_{\text{leaf}} = T_{\text{air}} + 5^{\circ}\text{C}$	32	95	100
iii) $T_{\text{leaf}} = T_{\text{air}} + 10^{\circ}\text{C}$	100	100	100
<b>b) Range of days in a year when estimated <math>T_{\text{leaf}} &gt; \text{mean } T_{50}</math></b>			
i) $T_{\text{leaf}} = T_{\text{air}}$	0	0	0-10
ii) $T_{\text{leaf}} = T_{\text{air}} + 5^{\circ}\text{C}$	0-3	0-38	10-94
iii) $T_{\text{leaf}} = T_{\text{air}} + 10^{\circ}\text{C}$	3-79	38-139	94-262

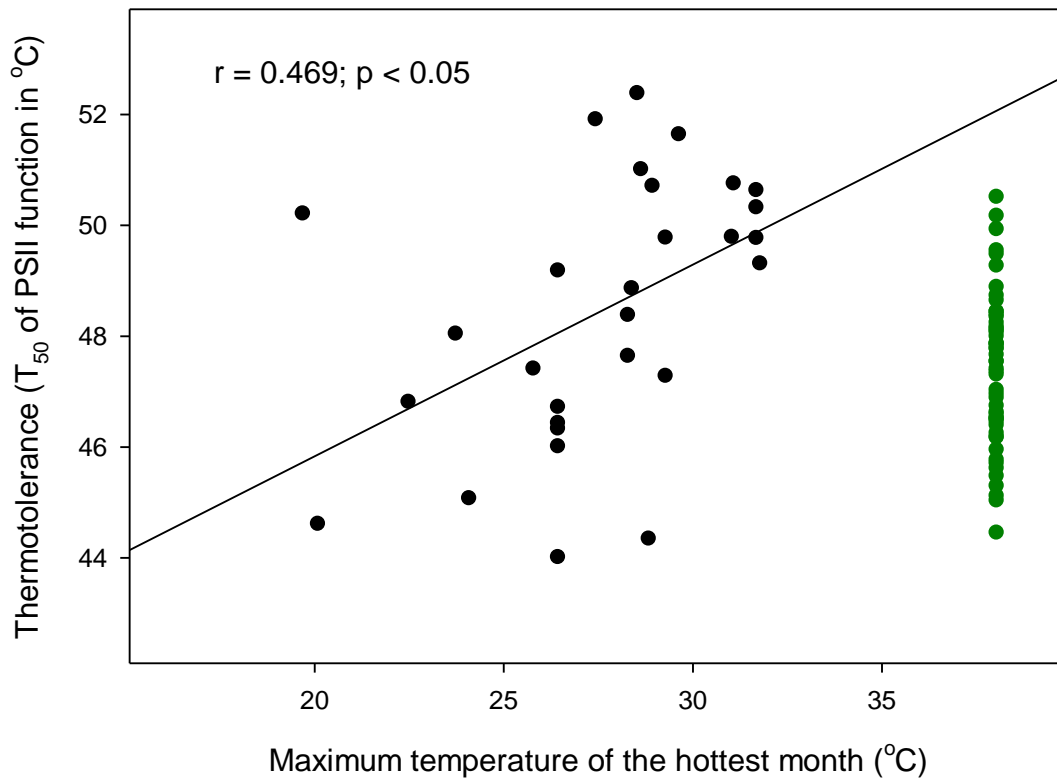


Figure 22: Relationship of thermotolerance ( $T_{50}$  for PSII function, where  $T_{50}$  is the temperature when PSII function is 50% of controls) and the monthly average maximum air temperature of the warmest month. The habitat temperature data were obtained from a high resolution global dataset (Mark et al. 2002). Each data point represents a single species for which data for thermotolerance ( $T_{50}$  for PSII function estimated by directly comparable methodologies). Only woody species were included in this data set. The green points represent the present study. The location of growth was used as the location to determine maximum air temperature. The data include 11 independent studies covering 29 woody species (excluding the present study).

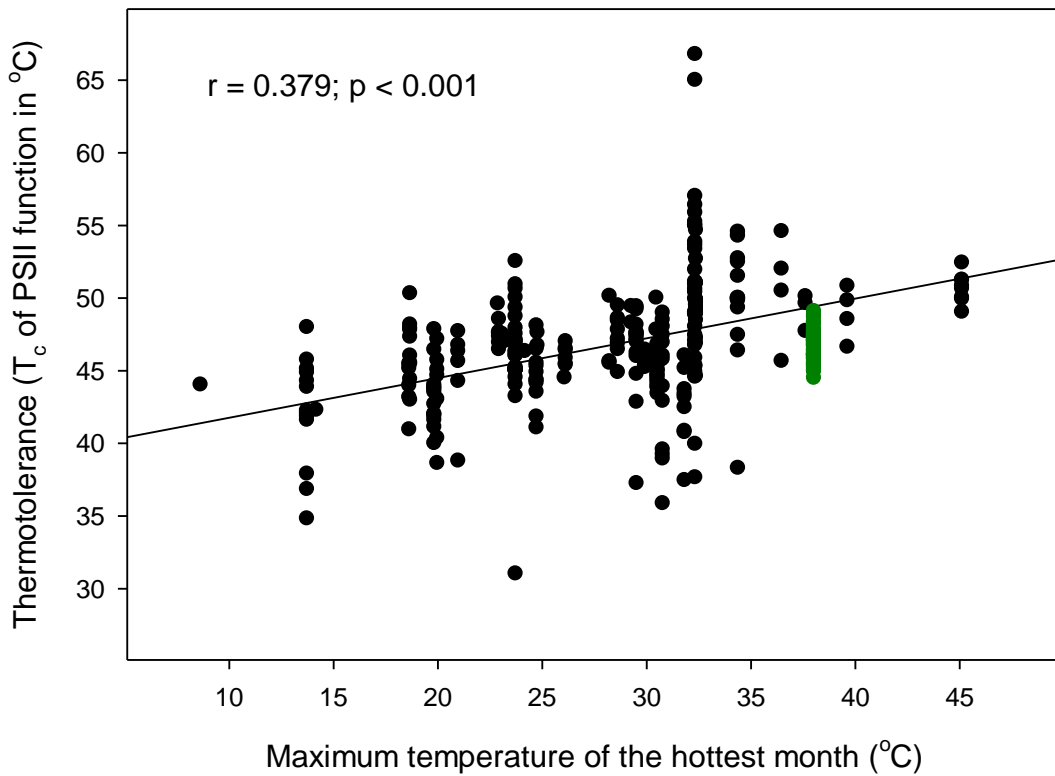


Figure 23: Relationship of thermotolerance ( $T_c$  for PSII function, where  $T_c$  is the critical temperature when a rapid rise in fluorescence is seen) and the monthly average maximum air temperature of the warmest month. The habitat temperature data were obtained from a high resolution global dataset (Mark et al. 2002). Each data point represents a single species for which data for thermotolerance ( $T_c$  for PSII function estimated by directly comparable methodologies). Only woody species were included in this data set. The green points represent the present study. The  $T_{50}$  values were converted into  $T_c$  for the present study based on the relationship given in Figure 29. The location of growth was used as the location to determine maximum air temperature. The data include 23 independent studies covering 337 woody species.

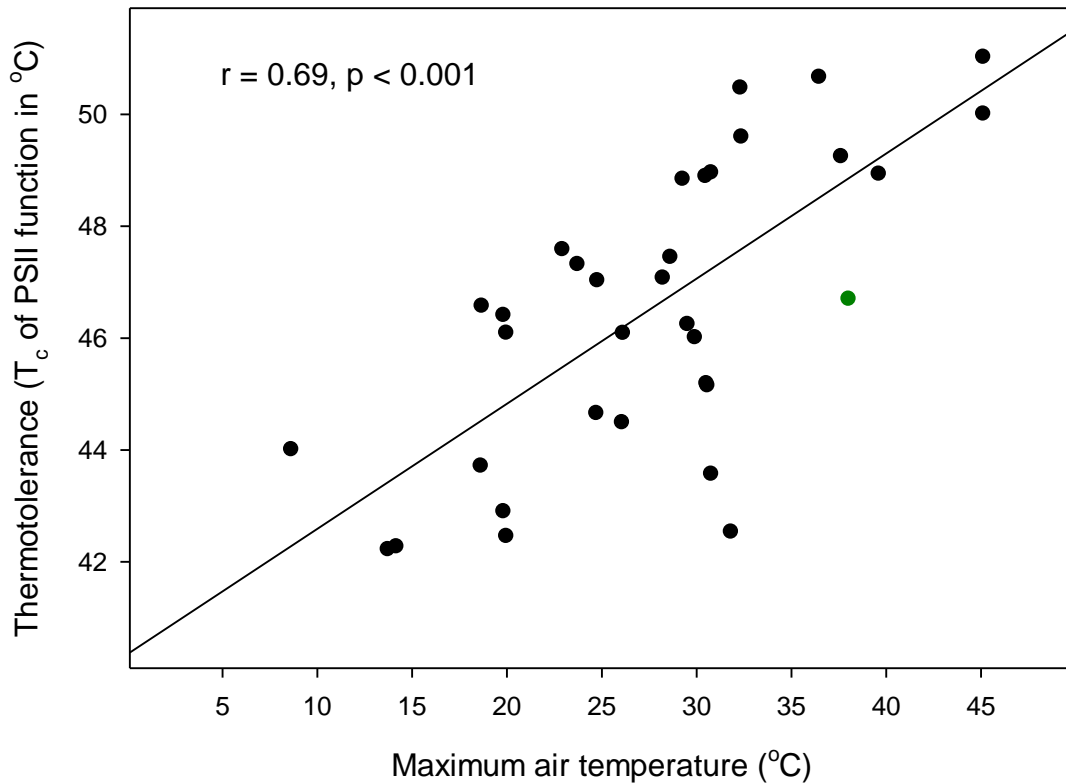


Figure 24: The relationship of mean thermotolerance ( $T_c$  for PSII function, where  $T_c$  is the critical temperature when a rapid rise in fluorescence is seen) at a site and the monthly average maximum air temperature of the warmest month at the site. The habitat temperature data were obtained from a high resolution global dataset (Mark et al. 2002). Each data point represents a single site for which data for thermotolerance ( $T_c$  for PSII function estimated by directly comparable methodologies). Only woody species were included in this data set. The green point represents the present study. The  $T_{50}$  values were converted into  $T_c$  for the present study based on the relationship given in Figure 29. The location of growth was used as the location to determine maximum air temperature. The data show site-specific means from 19 independent studies covering 271 woody species (excluding the present study).

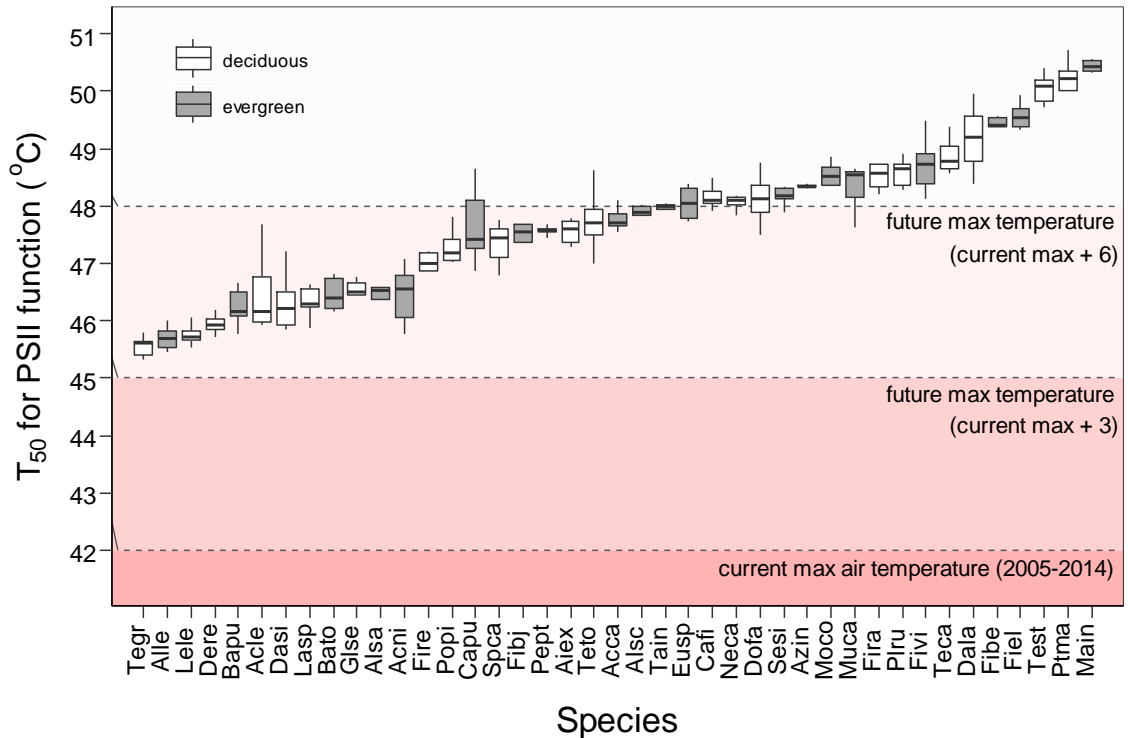


Figure 25: Thermotolerance ( $T_{50}$  for PSII function) for the 41-species examined. The difference between  $T_{50}$  and the three lines present a visual representation of the thermal safety margin for these species under current and future climates. The three lines represent: Current maximum daily temperatures experienced during 10 years (2005-2014; data from GHCN - Global Historical Climatology Network daily, Version 3.22); Current maximum temperatures +3°C; and Current maximum temperatures +6°C. Boxes represent the upper 75 and lower 25 percentiles, the central line represents the median, the whiskers are largest/lowest observation less than or equal to upper hinge + 1.5 x inter quartile range. Grey boxes represent evergreen and open boxes deciduous species. Details for the species are provided in Table 13.

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## 7. Supplementary material

### 7.1 Supplementary data for Chapter 2

Table 12: Variation in leaf mass per area (LMA). Results from a mixed model ANOVA with season (hot-dry and cool-wet) and leaf habit (evergreen and deciduous) as fixed effects and species as a random effect nested within leaf habit.

<b>Effect</b>	<b>df</b>	<b>MS</b>	<b>F</b>	<b>p</b>
Season	1	101.9	1.19	0.285
Leaf habit	1	6976.0	81.352	< 0.001
Season X leaf habit	1	80.2	0.94	0.342
Species (leaf habit)	27	2020.3	23.56	< 0.001
Error	27	85.8		

Table 13: List of species used in the study with family and whether or not the species is native to the region of study. If not native, the native regions have been mentioned.

<b>Code</b>	<b>Species name</b>	<b>Family</b>	<b>Origin</b>
Act	<i>Acacia catechu</i> (L.f.) Willd.	Leguminosae	Native
Acl	<i>Acacia leucophloea</i> (Roxb.) Willd.	Leguminosae	Native
Anl	<i>Acacia nilotica</i> (L.) Delile	Leguminosae	Tropical Africa
Ali	<i>Ailanthus excelsa</i> Roxb.	Simaroubaceae	Native
All	<i>Albizia lebbek</i> (L.) Benth.	Leguminosae	Native
Als	<i>Albizia saman</i> (Jacq.) Merr.	Leguminosae	Malayasia
Cba	<i>Alstonia scholaris</i> (L.) R. Br.	Apocynaceae	Native
Nem	<i>Azadirachta indica</i> A.Juss.	Meliaceae	Native
Bap	<i>Bauhinia purpurea</i> L.	Leguminosae	Native
Bat	<i>Bauhinia tomentosa</i> L.	Leguminosae	Native
Cpl	<i>Caesalpinia pulcherrima</i> (L.) Sw.	Leguminosae	Native
Cft	<i>Cassia fistula</i> L.	Leguminosae	Native
Leg	<i>Dalbergia lanceolaria</i> L.f.	Leguminosae	Native
Dal	<i>Dalbergia sissoo</i> DC.	Leguminosae	Native
Der	<i>Delonix regia</i> (Hook.) Raf.	Leguminosae	Madagascar
Dol	<i>Dolichandrone falcata</i> (Wall. ex DC.) Seem.	Bignoniaceae	Native
Ecl	<i>Eucalyptus</i> sp.	Myrtaceae	Australia
Ben	<i>Ficus benghalensis</i> L.	Moraceae	Native
Bnj	<i>Ficus benjamina</i> L.	Moraceae	Native
Fel	<i>Ficus elastica</i> Roxb. ex Hornem.	Moraceae	Native
Frc	<i>Ficus racemosa</i> L.	Moraceae	Native
Rel	<i>Ficus religiosa</i> L.	Moraceae	Native
Fvr	<i>Ficus virens</i> Aiton	Moraceae	Native
Gli	<i>Gliricidia sepium</i> (Jacq.) Walp.	Leguminosae	Central America
Lag	<i>Lagerstroemia speciosa</i> (L.) Pers.	Lythraceae	Native
Lle	<i>Leucaena leucocephala</i> (Lam.) de Wit	Leguminosae	Mexico
Man	<i>Mangifera indica</i> L.	Anacardiaceae	Native
Mop	<i>Morinda coreia</i> Buch.-Ham.	Rubiaceae	Native
Mun	<i>Muntingia calabura</i> L.	Muntingiaceae	Jamaica
Neo	<i>Neolamarckia cadamba</i> (Roxb.) Bosser	Rubiaceae	Native
Pph	<i>Peltophorum pterocarpum</i> (DC.) K.Heyne	Leguminosae	South-east Asia
Plu	<i>Plumeria rubra</i> L.	Apocynaceae	Central America
Mip	<i>Pongamia pinnata</i>	Leguminosae	Native
Ptm	<i>Pterocarpus marsupium</i> Roxb.	Leguminosae	Native
Ses	<i>Senna siamea</i> (Lam.) H.S.Irwin & Barneby	Leguminosae	Native

Table 13 continued...

<b>Code</b>	<b>Species name</b>	<b>Family</b>	<b>Nativity</b>
Spt	<i>Spathodea campanulata</i> P.Beauv.	Bignoniaceae	Africa
Tam	<i>Tamarindus indica</i> L.	Leguminosae	Tropical Africa
Tec	<i>Tecoma stans</i> (L.) Juss. ex Kunth	Bignoniaceae	Central America
Tct	<i>Tectona grandis</i> L.f.	Lamiaceae	Native
Trj	<i>Terminalia arjuna</i> (Roxb. ex DC.) Wight & Arn	Combretaceae	Native
Ter	<i>Terminalia catappa</i> L.	Combretaceae	Native

Table 14: Leaf phenology and tree heights. Species code (Sp - details in Table 13), Leaf habit (LH: E- evergreen, D - deciduous), Height of trees (range in m), deciduousness index (DI, %), month of peak leaf flushing (FL), month of peak leaf senescence (SEN). NA indicates phenology not monitored. SE - standard error, n - sample size.

Sp	Leaf Habit	n	Height	DI (%)	SE	FL	SEN
Act	E	8	3-5	5.31	0.47	Apr	Dec
Acl	D	10	5-8	17.08	0.26	May	Jan
Anl	E	5	8-12	10.83	2.04	Apr	Mar
Ali	D	10	10-15	27.08	0.62	Apr	Jan
All	E	10	10-20	8.58	0.55	Mar	Dec
Als	E	10	10-15	0.83	0	May	Dec
Cba	E	10	8-12	2.42	0.19	Apr	Feb
Nem	D	10	10-15	13.67	1.07	Apr	Jan
Bap	E	10	6-12	6.67	0.37	Apr	Jan
Bat	E	10	3-5	12.17	0.62	Jun	Jan
Cft	D	10	8-12	18.08	1.92	Apr	Dec
Cpl	E	10	5-8	12.33	0.3	Apr	Dec
Leg	D	4	10-15	7.71	0.52	Jun	May
Dal	D	10	8-12	13.04	0.82	May	Jan
Del	D	10	10-15	18.83	0.43	Mar	Jan
Dol	D	NA	5-8	--	--	--	--
Ecl	E	10	10-15	1.17	0.31	Apr	Jan
Ben	E	10	10-15	3.92	0.35	Jun	Apr
Bnj	E	10	10-15	0.92	0.34	Jan	May
Fls	E	4	10-15	0.83	0.41	Jan	Jan
Fir	D	10	10-15	10.33	0.42	Oct	Sep
Rel	D	10	10-15	9.17	0.33	Jun	Apr
Fvr	E	6	10-15	1.8	0.14	Apr	Dec
Gli	D	10	10-15	33.67	0.55	May	Jan
Lag	D	10	8-12	30.25	0.48	May	Apr
Lle	D	10	10-15	31	1.05	May	Jan
Man	E	10	10-15	2.08	0.14	May	Dec
Mop	E	10	8-10	8.83	0.36	Apr	Dec
Mun	E	10	5-8	2	0.31	Apr	Apr
Neo	D	NA	10-15	--	--	--	--
Ppe	E	10	10-15	15.25	0.51	Apr	Dec
Plu	D	10	3-5	15.75	0.61	Apr	Dec
Mip	D	4	8-10	11.46	0.52	Apr	Feb
Ptm	D	NA	10-15	--	--	--	--
Ses	E	10	10-15	10.75	0.49	Apr	Dec
Spt	D	6	10-15	11.94	2.45	Apr	Jan
Tam	E	4	10-15	9.79	0.4	May	Jan
Tec	D	10	8-10	15.29	0.8	Jan	Apr
Tct	D	10	10-15	57.75	0.43	Mar	Dec

Table 14 continued...

<b>Sp</b>	<b>Leaf Habit</b>	<b>n</b>	<b>Height</b>	<b>DI (%)</b>	<b>SE</b>	<b>FL</b>	<b>SEN</b>
Ter	D	10	5-8	7.75	0.4	Jun	Apr
Arj	D	NA	10-15	--	--	--	--

Table 15: Leaf traits Species code (Sp - details in Table 13), leaf mass per area - dry season (disc LMA-d, g·m<sup>-2</sup>), leaf size - dry season (LA-d, cm<sup>2</sup>), leaf mass per area - rainy season (disc LMA-r, g·m<sup>-2</sup>), leaf size - rainy season (LA-r, cm<sup>2</sup>), T<sub>50</sub> - hot dry season (T<sub>50-D</sub>, °C), T<sub>50</sub> - rainy season (T<sub>50-R</sub>, °C). NA indicates that healthy mature leaves for that species not available, or otherwise not measured. SE - standard error, n - sample size.

Sp	LMA-d	SE	LA-d	SE	n	LMA-r	SE	LA-r	SE	n	T50-d	SE	n	T50-r	SE	n
Acca	NA	--	36	2.2	--	45	1.4	NA	--	5	47.8	0.1	6	NA	--	--
Acle	48.3	0.9	36.9	2.4	5	47.4	1.3	42	8.6	5	46.5	0.3	5	46.2	0.2	6
Acni	NA	--	6.2	0.4	--	50.2	3.2	NA	--	5	46.4	0.2	5	NA	--	--
Aiex	82.8	1	535.3	43.3	5	62.4	3.8	996.9	152.7	5	47.4	0.5	6	48	0.8	6
Alle	NA	--	NA	--	--	33.8	1.8	205.1	51.3	2	NA	--	--	45.7	0.1	6
Alsa	64.4	1.4	82.2	2.7	5	76.3	1.8	59.7	8	5	46.5	0.3	5	45	0.2	6
Alsc	44.2	0.6	45.9	1.8	5	83.3	6.9	56.9	6.8	5	47.9	0.1	6	48	0.2	6
Azin	64.4	5.6	61.4	2.1	5	91.2	3.1	140.4	35.5	4	48.4	0.1	6	50	0.3	6
Bapu	54.3	2.7	130.8	11.3	5	45.9	4.4	109.7	8.4	5	46.2	0.1	6	45.2	0.1	6
Bato	53.2	1.3	14.6	0.6	5	52.9	6.3	15.7	1.6	5	46.7	0.4	6	46.4	0.4	6
Cafi	62.1	1.5	524.8	20.8	5	NA	--	530.8	55.5	--	48.2	0.1	6	45.7	0.1	4
Capu	30.1	2.3	416.8	18.3	5	33.1	0.3	408	24.6	5	47.7	0.3	6	45.2	0.4	6
Dala	60.5	2.9	100.1	5.8	5	62.2	3.3	42.3	3.2	5	49.2	0.5	3	48.7	0.5	3
Dasi	68.9	1.2	55.9	2.3	5	80.9	2.6	61.8	2.9	5	46.2	0.3	6	45.5	0.4	6
Dere	64.5	2	247.7	9.9	5	66.8	0.9	248	13.6	5	45.9	0.1	6	45.2	0.1	6
Dofa	104.5	2.9	41.8	1.7	5	NA	--	NA	--	--	48.1	0.2	6	48.1	0.3	6
Eusp	152.5	4.6	32.7	1.5	5	164.2	7.4	37.1	2.3	5	47.8	0.3	6	45.4	0.1	6
Fibe	111.8	3.1	122	6.3	5	118.7	6.9	73.5	5.7	5	49.5	0.2	6	47.7	0.1	6
Fibj	79.3	1.3	13.5	0.3	5	95.9	5.1	14.7	0.7	5	47.5	0.2	6	48.3	0.2	6
Fiel	185	4.6	193.3	8.5	5	171.6	7.9	151.7	22.5	5	49.5	0.2	6	48.6	0.3	6
Fira	101.1	6.5	43.3	1.7	5	NA	--	NA	--	--	48.6	0.2	6	NA	--	--
Fire	97.6	3.1	138.8	4	5	83.1	1.2	89.7	10.7	5	46.9	0.2	6	46	0.1	6
Fivi	127.9	2.3	48.5	1.2	5	136.2	6.9	43.8	3	5	48.7	0.2	6	48.3	0.1	6
Glse	50.6	1.2	104.5	3.9	5	40.8	1.6	151	7.8	5	46.5	0.1	6	45.2	0.4	6
Lasp	108.9	6	124.6	9.7	5	88	1.7	149.7	9.4	5	46.4	0.2	6	45.1	0.1	6
Lele	64.2	5.4	58.7	3	5	64.8	2.1	60	7.1	5	45.8	0.1	6	44.4	0.2	6
Main	107.1	1.8	97.6	4.1	5	113.6	5	82	2.6	5	50.5	0.1	6	48.5	0.1	6
Moco	87	1.4	49.6	1.9	5	102.5	4.3	49.9	13.1	5	48.4	0.2	6	48	0.2	6
Muca	66.3	2.8	29.9	1.2	5	56.5	3.1	35	2.3	5	48.3	0.2	6	46.3	0.1	6
Neca	NA	--	NA	--	--	42.6	1.9	201.7	32.6	5	48.1	0.1	4	NA	--	--
Pept	84	2.7	349.8	34.2	5	80.2	2.4	292	36	5	47.6	0	5	47.8	0.2	6
Plru	104	7.6	91.9	3.3	5	110.3	6	112.1	4.4	5	48.4	0.2	6	48	0.3	6
Popi	68.4	1.6	380.9	45.4	5	69.7	3.9	185	14.8	5	47.3	0.2	4	45.7	0.6	4
Ptma	103	2.1	42.1	1.6	5	NA	--	NA	--	--	50.1	0.3	4	NA	--	--
Sesi	97.4	2.5	163.2	5.9	5	92.5	7.6	202.7	9.7	5	48.2	0.1	6	47.1	0.1	5
Spca	60.5	6.3	NA	--	5	NA	--	319.9	36.3	--	47.3	0.2	6	48.5	0.1	6
Tain	51.3	1.6	13.9	0.8	5	53.4	3.3	12	2	5	48	0	4	46.9	0.3	4
Teca	78.5	2.5	178.2	8.9	5	NA	--	NA	--	--	48.9	0.1	6	46.3	0.2	5
Tegr	NA	--	NA	--	--	80	1.6	580.6	46.1	5	NA	--	--	45.5	0.2	6
Test	61.7	3	118.9	4.3	5	55.1	3.1	201.2	21.4	5	49.9	0.2	6	46.4	0.2	6
Teto	NA	--	NA	--	--	55	--	171	--	1	47.8	0.3	4	NA	--	--

Table 16: Seasonally separated relationship between leaf traits and thermotolerance. Relationship between thermotolerance ( $T_{50}$  for PSII function) and a) leaf mass per area ( $\text{g}\cdot\text{m}^{-2}$ ), and b) leaf area ( $\text{cm}^2$ ). These analyses were conducted for the hot dry season and the cooler monsoon season separately. Values for Pearson's correlation coefficient (log transformed LMA to meet assumptions of normality), and Spearman's rank correlation are shown (untransformed values of LMA).

		(Pearson)		(Spearman)	
	<b>n</b>	<b>r</b>	<b>p</b>	<b>R</b>	<b>p</b>
<b>a) leaf mass per area</b>					
dry season	35	0.41	0.013	0.41	0.015
rainy season	31	0.48	0.006	0.46	0.010
<b>b) Leaf area</b>					
dry season	36	0.07	0.685	0.01	0.994
rainy season	33	-0.11	0.560	-0.13	0.455

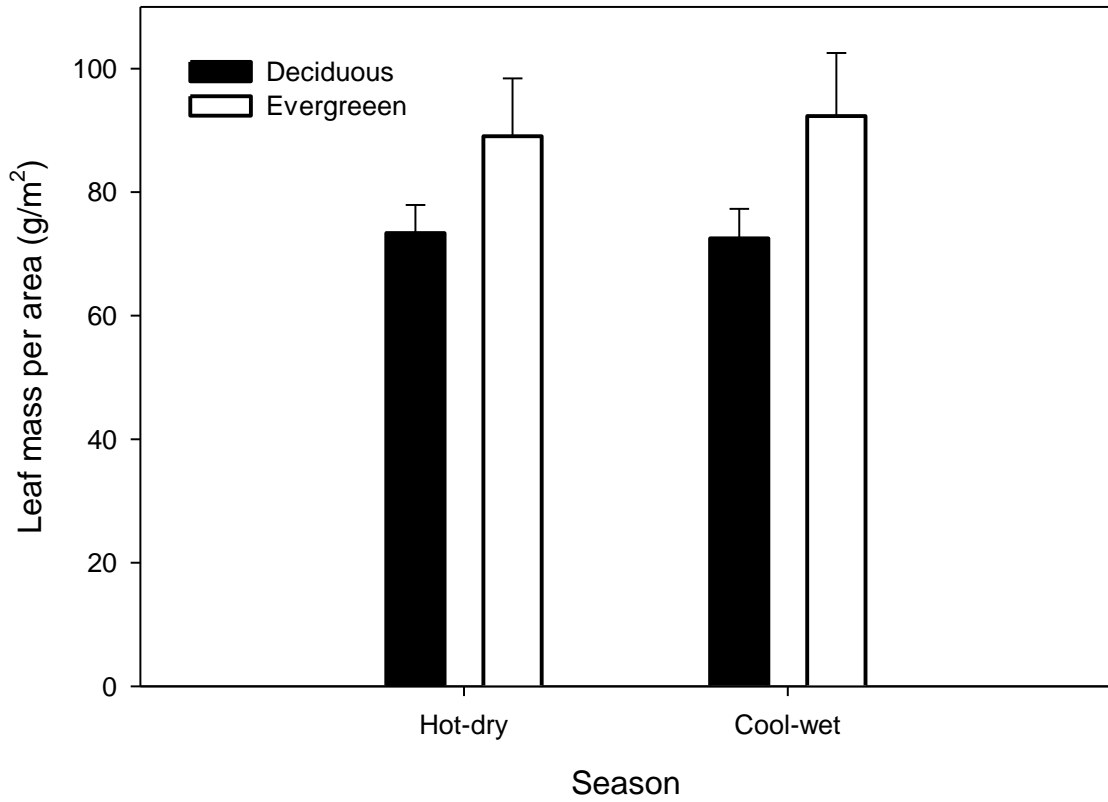


Figure 26: Seasonal variation in leaf mass per area (LMA). Results of a mixed model ANOVA. There is no difference in LMA in the two seasons, but evergreen species (white bars) have a slightly higher LMA compared to the deciduous species (black bars).



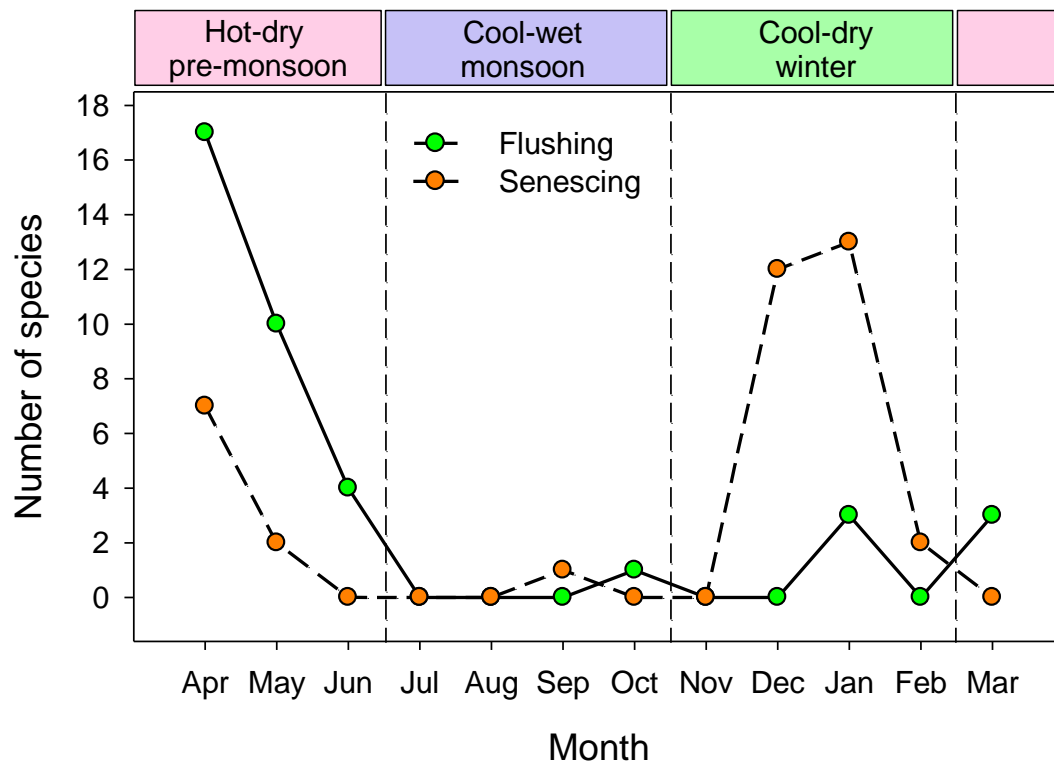


Figure 27: Leaf flushing and senescing patterns. The number of species in peak leaf flushing (green) and senescing (orange) for the 37 species for which leaf phenology was monitored between April 2014 and March 2015.

## 7.2 Supplementary data for Chapter 4

Table 17: Variation in thermotolerance (PSII function at 25°C, 47.5°C, 50°C) for 11 species under control (well-watered) and drought stressed conditions.

<b>Effect</b>	<b>df</b>	<b>MS</b>	<b>F</b>	<b>p</b>
Temperature	2	12.74	1284	<0.001
Drought	1	0.64	65	<0.001
Species	10	0.11	12	<0.001
Temperature x Drought	2	0.39	39	<0.001
Temperature x Species	20	0.03	3	<0.001
Drought x Species	10	0.01	1	0.624
Temperature x drought x Species	20	0.01	1	0.168

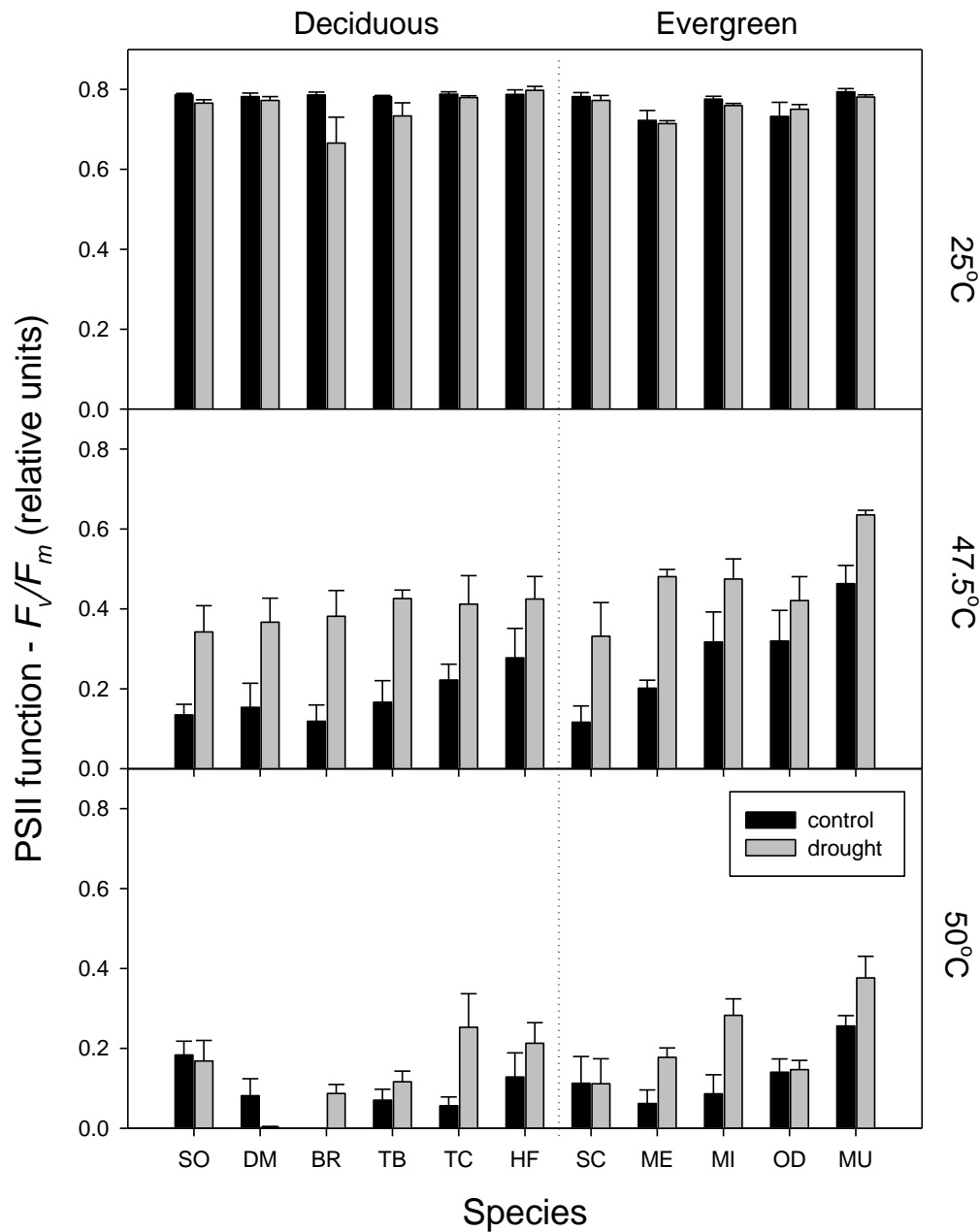


Figure 28: Effect of drought on thermotolerance of different species. The effect of drought stress on dark adapted chlorophyll *a* fluorescence ( $F_v/F_m$ ) at 25°C, 47.5°C, and 50°C. Black and grey bars represent control (well-watered), and drought stressed conditions, respectively. Error bars represent standard error (n = 5 - 6). Species names are provided in Table 8.

### 7.3 Supplementary data for Chapter 5

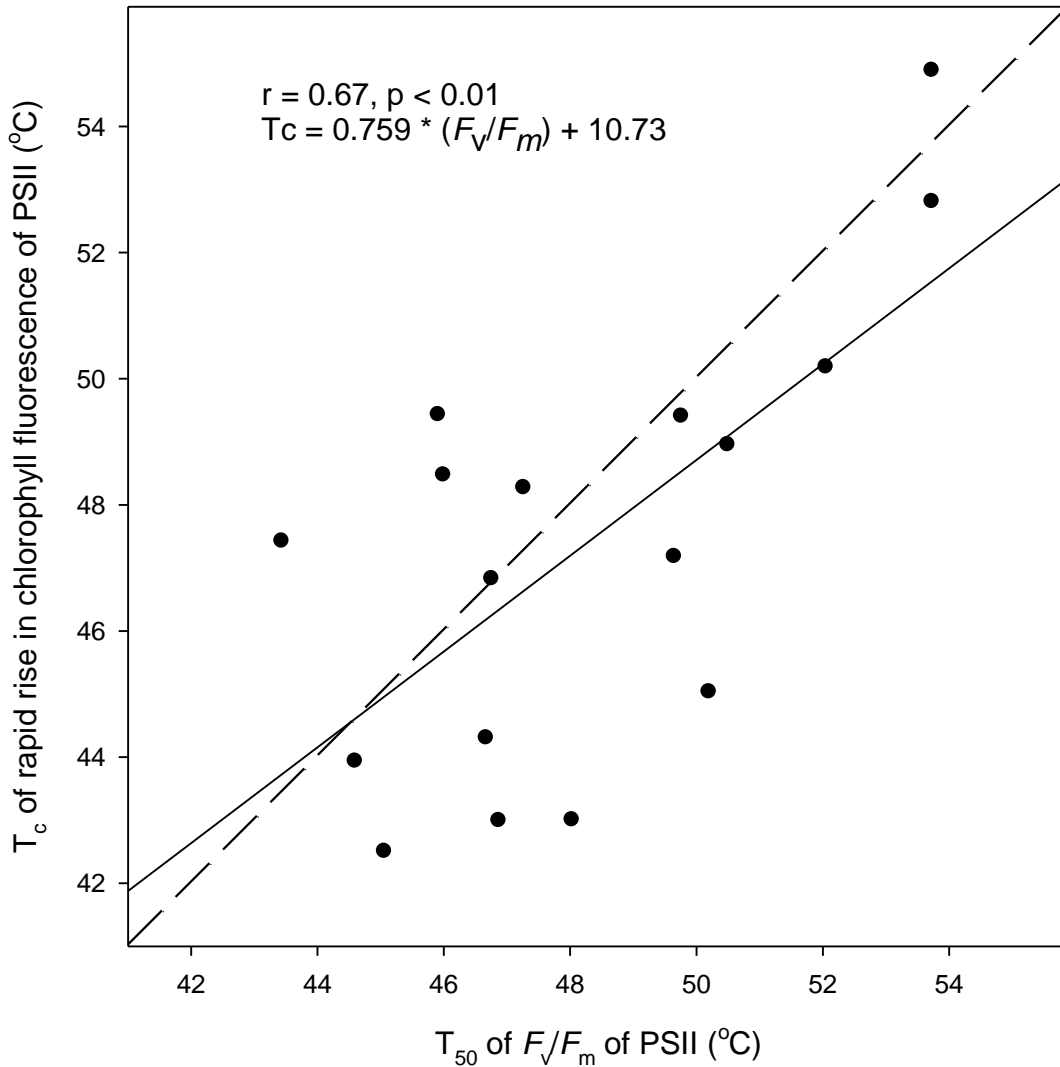


Figure 29: Relationship between T<sub>c</sub> of rapid rise of fluorescence of PSII as measured when heated at 1°C/min and T<sub>50</sub> of F<sub>v</sub>/F<sub>m</sub> of PSII as measured when treated at each temperature for 30 minutes. The data include 16 independent studies. As far as possible studies which had both measures were used, but this was not the case for 7 of the species. The linear equation of this relationship was used to convert T<sub>50</sub> of the present study into T<sub>c</sub> to compare with other studies.