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Foliar temperature tolerance of temperate and tropical evergreen rain forest trees of Australia

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Summary Australian rain forests extend from tropical climates in the north to temperate climates in the south, providing an opportunity to investigate physiological responses to temperature of both temperate and tropical species within the same forest type. Eight, rain forest canopy tree species were selected to cover the 33° latitudinal range of rain forests in eastern Australia. Temperature tolerance was measured in 6-year-old plants grown in a common environment, by exposing leaves to a series of high temperatures during late summer and a series of freezing temperatures during midwinter. Damage was evaluated based on chlorophyll fluorescence measurements made 2 h after exposure and by visual assessment of leaf damage made a week after exposure. Leaves of the tropical species were more heat tolerant and less frost tolerant than leaves of the temperate species, which is consistent with their climate distributions. In contrast, the temperature tolerance of the photosynthetic apparatus was unrelated to climate in a species' native habitat. However, the tropical species underwent significant photoinhibition during winter. All species maintained the integrity of the photosynthetic apparatus and avoided tissue damage over a similar span of temperatures (about 60 °C), reflecting the similar annual temperature ranges in Australia's temperate and tropical rain forests. Chlorophyll fluorescence measurements and visual assessment of leaf damage provided different estimates of the absolute and relative temperature tolerances of the species, thus emphasizing the importance of a direct assessment of tissue damage for determining a species' temperature tolerance.

Keywords: chlorophyll fluorescence, climate, frost tolerance, heat tolerance, latitude, leaf damage, photoinhibition.

Introduction

The physiological tolerance of plants to temperature is often used to model their distribution at the level of vegetation types (e.g., Box 1981). Woodward (1987) proposed a model that emphasized the importance of cold tolerance and the length of the growing season in determining the high latitude limits of forest types. The cost of cold tolerance in the high-latitude forest types was proposed to reduce their competitive ability under the warmer conditions at low latitudes. Recent vegetation-climate models (e.g., Box 1995, Neilson 1995) have continued to explain the distribution of forest types on the basis of tolerance of climatic extremes.

The frost tolerance of a species usually reflects the range of temperatures encountered throughout its natural distribution (Sakai and Larcher 1987). However, at the regional level, the frost tolerance of a tree species does not always correlate with its altitudinal distribution (Read and Hill 1988). In contrast, the high temperature tolerance of species is more complicated, and direct correlations between tolerance and distribution are often not apparent (Kappen 1981). For example, the heat tolerance of many alpine plants is higher than would be predicted from the climate of their native habitat, because their prostrate form decouples them from ambient conditions (Körner 1999). Furthermore, at the regional level, xeric species can have greater heat tolerances than mesic species (Hamerlynck and Knapp 1994).

Previous comparisons of temperature tolerance among species from contrasting climates have been based on responses of species growing in their native habitats (e.g., Kappen 1981, Sakai and Larcher 1987). Such comparisons ignore the ability of both warm and cool climate species to adjust their temperature tolerance when grown at different temperatures (e.g., Hamerlynck and Knapp 1994, Greer and Robinson 1995). Currently, there have been few direct comparisons of species from contrasting climates grown in the same environment (e.g., Read and Hope 1989). Not surprisingly, studies of frost tolerance have concentrated on temperate plantation species (e.g., Raymond et al. 1992, Calmé et al. 1994), whereas studies of heat tolerance have been much more diverse, including desert species (e.g., Downton et al. 1984), tropical crops (e.g., Yamada et al. 1996) and alpine species (e.g., Buchner and Neuner 2003). In addition, heat and frost tolerances have rarely been measured in the same species (but see Burr et al. 1993).

Comparisons among studies are hindered by the use of different techniques to assess temperature tolerance of leaves, including visual assessment (e.g., Bannister 1984), conductivity of leaked electrolytes (e.g., Hallam and Tibbits 1988) and chlorophyll fluorescence (e.g., Seemann et al. 1986). Direct comparisons of these techniques have shown good correlations between chlorophyll fluorescence and electrolyte leak-

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age with visible leaf damage (Raymond et al. 1992, Lindgren and Hällgren 1993). However, chlorophyll fluorescence, an indicator of photosynthetic performance, has been found to overestimate frost tolerance (Neuner and Buchner 1999) and to underestimate heat tolerance of leaf tissue (Bigras 2000). Additionally, comparisons of temperature tolerances among studies are difficult because measurements are affected by seasonal timing (Burr et al. 1993) and water stress (Ladjal et al. 2000).

Australia's rain forests provide an opportunity to investigate the physiological responses to temperature of both temperate and tropical species. Rain forests in Australia occur across a latitudinal range of 33°, which includes cool-temperate, warm-temperate, subtropical and tropical forests. These forests have a disjunct distribution along the eastern margin of Australia, being restricted to areas with a high annual rainfall (> 1500 mm) and low fire frequency (Webb and Tracey 1994, Specht and Specht 1999).

Depending on latitude of origin, Australian rain forest tree species differ in several physiological responses to temperature. Species from higher latitudes have maximum net photosynthesis at lower acclimation temperatures than species from lower latitudes (Hill et al. 1988, Cunningham and Read 2003*b*) and maintain maximum rates of photosynthesis over a wider span of temperatures (Read 1990, Cunningham and Read 2003*b*). Similarly, temperate species attain maximum net photosynthetic rates at lower growth temperatures (the temperature at which leaves are developed) than tropical species, but maintain near maximum rates of photosynthesis over a wider span of temperatures (Cunningham and Read 2002). Furthermore, temperate species achieve maximum growth rates at lower temperatures than tropical species (Cunningham and Read 2003*a*). Comparisons of the responses to extreme temperatures among temperate and tropical rain forest trees have so far been restricted to the genus *Nothofagus*, in which frost resistance is greatest in the temperate species (Read and Hope 1989).

The present study aimed to further our understanding of temperature tolerance in plants by investigating both heat and cold tolerance in a broad range of species (different genera) of the same growth form (evergreen, canopy rain forest trees), from a wide range of climates (temperate to tropical), under the same temperate climate without water or nutrient stress. A secondary aim was to compare chlorophyll fluorescence, which can be measured in hours, with visible symptoms of leaf damage, which can take several weeks to develop, as a means to assess tolerance to high and low temperatures.

Material and methods

Species selection

Eight tree species were selected to cover the latitudinal range of rain forests in eastern Australia. Distributional ranges and collection sites of the species are given in Table 1 and their climate profiles are given in Table 2. Two dominant species were selected from each of the four rain forest types (cool-temperate, warm-temperate, subtropical and tropical) defined by Webb (1968). Canopy dominant species were chosen, as it can be assumed that these are exposed to the macroclimate in their native environment. All species were evergreen, ensuring that their leaves are exposed to the entire annual climatic cycle. Species from different families were chosen where possible to minimize the confounding effects of phylogenetic relatedness.

Growth conditions

Seedlings of all species were from natural populations, col-

Table 1. Distributional ranges and collection sites for the study species.

Species	Distributional range		Collection site		
	Latitude (S)	Altitude (m)	Latitude (S)	Longitude (E)	Altitude (m)
Temperate					
Eucryphia lucida (Labill.) Baill. (Eucryphiaceae)	41–43.5°	5–1000	41°10′	144°57′	140
Nothofagus cunninghamii (Hook.) Oerst. (Fagaceae)	37–43.5°	0–1440	41°9′	145°01′	180
Tristaniopsis laurina (Sm.) Wilson & Waterhouse (Myrtaceae)	25.5–38°	5–1035	37°42′	147°22′	150
Acmena smithii var. *smithii* (Poir.) Merrill & Perry (Myrtaceae)	24.5–39°	0–1270	37°25′	149°49′	200
Tropical					
Sloanea woollsii F. Muell. (Elaeocarpaceae)	26–32°	20–1200	30°43′	152°43′	60
Heritiera trifoliolata (F. Muell.) Kosterm. (Sterculiaceae)	17–30°	10–1075	28°36′	152°43′	540
Castanospermum australe Cunn. & C. Fraser ex Hook. (Fabaceae)	12.5–30°	5–1150	26°38′	153°38′	40
Alstonia scholaris (L.) R. Br. (Apocynaceae)	10.5–22°	0–1300	16°13′	145°52′	20

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Table 2. Climate profiles for the study species. Values are means ± standard errors (with ranges in brackets) of the temperature profiles produced by ANUCLIM 5.0 (Houlder et al. 1999) from *n* site locations. Species are presented in order from highest to lowest latitudinal origin. For details of the climate analysis see Cunningham and Read (2003*a*).

Species	*n*	Maximum temp. hottest month (°C)	Minimum temp. coldest month (°C)	Annual temperature range (°C)	Mean annual precipitation (mm year^{-1})
Temperate					
E. lucida	112	18.8 ± 0.1 (14.5–21.3)	2.2 ± 0.1 (–1.0–5.0)	16.0 ± 0.1 (13.7–21.1)	2072 ± 52
N. cunninghamii	354	19.5 ± 0.1 (12.9–29.9)	1.6 ± 0.1 (–1.4–6.4)	17.9 ± 0.1 (12.3–26.9)	1764 ± 28
T. laurina	137	26.4 ± 0.2 (20.8–31.0)	4.5 ± 0.2 (0.2–9.5)	21.9 ± 0.2 (16.1–28.3)	1318 ± 34
A. smithii	291	26.5 ± 0.1 (21.7–32.2)	5.3 ± 0.2 (–0.2–14.3)	21.3 ± 0.2 (14.6–29.5)	1320 ± 24
Tropical					
S. woollsii	140	26.4 ± 0.1 (22.6–30.4)	3.7 ± 0.2 (–0.2–10.2)	22.7 ± 0.2 (17.0–27.0)	1395 ± 27
H. trifoliolata	98	27.7 ± 0.2 (21.2–30.9)	7.3 ± 0.3 (1.0–14.3)	20.4 ± 0.3 (14.6–26.2)	1750 ± 64
C. australe	123	29.5 ± 0.2 (25.6–33.5)	10.3 ± 0.4 (2.0–18.0)	19.2 ± 0.3 (13.3–26.6)	1655 ± 59
A. scholaris	61	30.7 ± 0.2 (25.1–35.0)	14.3 ± 0.4 (5.7–21.5)	16.4 ± 0.4 (9.9–26.5)	1978 ± 94

lected as recently germinated seedlings or, in the case of the two tropical species *Alstonia scholaris* (L.) R. Br. and *Castanospermum australe* Cunn. & C. Fraser ex Hook., raised from seed. Seedlings were housed in greenhouses at Monash University, Melbourne (37°56′ S 114°31′ E) for six years before the experiment. The greenhouses were heated during the cooler months to ensure that night temperatures did not fall below 10 °C. Seedlings were grown in pots containing sandy loam soil and repotted regularly to ensure that plants had adequate root systems. Pots were watered to field capacity every 2–7 days depending on the time of year and supplied every 14 days with a commercial fertilizer solution providing 240 mg l^{-1} of nitrogen, 49 mg l^{-1} of potassium and 80 mg l^{-1} of phosphorus per application.

Estimation of heat tolerance

The experiment began in summer (February 2002), with six plants of each species randomly arranged across two greenhouses. Heat tolerance of leaves was determined during April 2002 (early autumn) based on chlorophyll fluorescence and visible damage. During the 2 weeks before measurements were taken, mean maximum temperature within the greenhouses was 26.2 ± 1.5 °C, ranging between 18 and 37 °C, and these temperatures are representative of summer temperates in southern Australia. Six plants per species were sampled over a 4-day period, when the maximum temperature within the greenhouses ranged from 31 to 37 °C. Each morning, 12 plants were randomly selected and 10 fully expanded leaves were sampled from each plant, immediately stored in zip lock bags with moist paper and placed in darkness. Chlorophyll fluorescence of leaves was measured in the laboratory at room temperature with a PAM-2000 pulse-amplitude modulation fluorometer (Heinz-Walz, Effeltrich, Germany). Leaves were dark-acclimated for 30 min before measurement. All measurements were made in the saturation pulse mode using the standard procedures described in the PAM-2000 manual (Heinz-Walz 1993). Initial fluorescence (F_o) was excited with a dim, non-actinic light (3 µs pulses at a frequency of 600 Hz and a wavelength of 655 nm) and maximum fluorescence (F_m) was induced by an 800 ms pulse of intense white light (> 4000 µmol m^{-2} s^{-1}). The ratio of variable to maximum fluorescence (F_v/F_m) was determined as: $F_v/F_m = 1 - F_o/F_m$. The F_v/F_m ratio provides a good estimate of the maximum quantum yield of PSII photochemistry in dark-acclimated leaves (Björkman and Demmig 1987) and is a good indicator of the integrity of the photosynthetic apparatus. After the fluorescence measurements, each leaf was individually sealed in a zip lock bag with moist paper, which was sealed inside a second zip lock bag. Five water baths were used to produce the following range of temperatures: 33, 36, 39, 42, 45, 48, 51, 54, 57 and 60 °C. Each day the 10 temperature treatments were randomly allocated to water baths, with five temperature treatments run during the morning and five during the afternoon. A single, bagged leaf from each replicate plant was placed in each water bath and incubated for 30 min. After the heat treatment, leaves were allowed to cool for 2 h at room temperature before the F_v/F_m ratio was remeasured. The above method was chosen over the temperature-dependent increase in fluorescence measured in single leaves (T–F_o curves) used in other studies (e.g., Knight and Ackerly 2002) because it allows the effect of a single temperature to be measured without the confounding effect of previous treatment temperatures, and it also allows direct comparison with visible damage in the same leaf. Heat-treated

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leaves were wrapped in moist paper towel, sealed in zip lock bags and stored in darkness at room temperature. After 10 days, the amount of damage to the leaves was estimated to the nearest 5% by placing a transparent grid over the leaf (cell size was 2 mm–1 cm depending on the leaf size of a species) and counting the number of grid cells containing damaged and undamaged tissue.

Estimation of frost tolerance

After the heat tolerance experiment, plants were grown outside to expose them to the ambient conditions of a temperate winter. Frost tolerance of leaves was estimated during winter (mid-July 2002) in the same way as described for the estimation of heat tolerance, i.e., by chlorophyll fluorescence measurements before and after the treatment, and visual assessments of damage 10 days after the treatment. During the 2 weeks before the measurements, the mean minimum temperature was 7.2 ± 0.3 °C, ranging between 4.6–10.6 °C. At this time, plants of the tropical species *Alstonia scholaris* had dropped several leaves and the remaining leaves were chlorotic. Frost tolerance of leaves was determined over a 6-day period from the same six plants per species as sampled in the heat tolerance experiment. A freezer was used to expose leaves to the following range of temperatures: 9, 6, 3, 0, –3, –6, –9, –12, –15 and –18 °C (± 0.2 °C). Each temperature treatment was run once in the freezer, so two polystyrene tubs were placed in the freezer to produce replicate treatments, with leaves of three individuals per species placed in each tub. Temperatures within the tubs were monitored with digital thermometers. Leaves, sealed in zip lock bags with moist paper towel, were placed in the freezer at an initial temperature of 9 °C. The temperature was lowered, at a rate of not more than 4 °C h^{-1}, until the treatment temperature was reached. Leaves were held at the treatment temperature for 30 min, which is representative of the time leaves are exposed to freezing temperatures in the field, and the temperature was then raised to 9 °C at a rate of not more than 4 °C h^{-1}.

Data analysis

The F_v/F_m ratios measured after the temperature treatments were calculated as percentages of initial F_v/F_m values. Both the percentage reduction in initial F_v/F_m and percent visible leaf damage showed a sigmoidal relationship with treatment temperature. The responses that increased with increasing temperature (percentage reduction in initial F_v/F_m at low temperatures and percent damage at high temperatures) were regressed with a Gompertz equation (Equation 1) and the responses that decreased with increasing temperature (percentage reduction in initial F_v/F_m at high temperatures and percent damage at low temperatures) were regressed with a logistic equation (Equation 2).

$$y = d + a\exp\left(-\exp\left(b\left(T - c\right)\right)\right) \quad (1)$$

$$y = d + \frac{a}{\left(1 + \exp\left(b - cT\right)\right)} \quad (2)$$

where y is the percentage reduction in initial F_v/F_m or percent damage, T is the treatment temperature and a, b, c and d are fitting parameters. From these regressions, the temperatures that caused a 50% reduction in initial F_v/F_m (FT_{50}) or 50% damage to the leaf (LT_{50}) were estimated. The temperature span (T_{span}) between the hot and cold limits of a response was then determined from the range between hot and cold T_{50} values for individual plants. However, because individual plants were subreplicates in the frost experiment (there being only two replicate treatments) the same groupings of subreplicate plants as in the frost experiment were used to calculate two values of temperature span for each species. Variables from the temperature response curves were then analyzed as a randomized block design with water bath in the heat tolerance experiment or tub in the frost tolerance experiment used as the blocking variable. A Tukey adjustment was used on probability values for all pairwise comparisons of means. A critical value of $\alpha = 0.05$ was used for all tests of significance. The seasonal change in chlorophyll fluorescence between autumn and winter was calculated from the pretreatment values of F_v/F_m measured in the heat and frost tolerance experiments. The difference between chlorophyll fluorescence measured in autumn and winter was tested by one-way repeated measures analysis of variance (ANOVA) within a species because the same six plants were used. Relationships among species means of T_{50} values and appropriate climate variables were determined by linear regression. Trends in the T_{50} values were observed regardless of the source of the climate profiles (mean climate profiles of the species or the climate profiles of the collection sites), so only regressions with the mean climate variables are presented.

Results

Leaves of the tropical species tended to have a higher heat tolerance than leaves of the temperate species (Table 3). However, the temperate species *Eucryphia lucida* from the coldest climate and the tropical species *Alstonia sholaris* from the hottest climate both had a 50% reduction in initial F_v/F_m at 50 °C. This meant there was no significant relationship between the high temperature causing a 50% reduction in initial F_v/F_m and the maximum temperature of the hottest month in the species' native habitat (Figure 1A). In contrast, there was a strong relationship between the high temperature causing 50% visible leaf damage and the temperature of the warmest month in the species' native habitat (Figure 1B), with species from the warmest native habitats having highest heat tolerances.

There was a significant reduction in the chlorophyll fluorescence (F_v/F_m) of untreated leaves of all species between April (early autumn) and July (midwinter; Table 4). This winter photoinhibition of leaves was most pronounced in the tropical species, and therefore was strongly related to the temperature minimum of the coldest month in a species' native habitat (Figure 2A). Although the tropical species *Alstonia scholaris* had a large influence on this relationship ($L = 0.6$), repeating the regression without this species nevertheless produced a significant linear relationship ($F = 13.1$, $P = 0.02$). As a result

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Table 3. Temperatures causing a 50% reduction in initial F_v/F_m (FT_{50}) and 50% visible leaf damage (LT_{50}). Values are means of six runs for the hot treatment, and two runs for the cold treatment. Temperature span (T_{span}) with standard deviations are given in brackets. The results of randomized block ANOVAs comparing species are given. Letters denote nonsignificant differences among means. Abbreviation: Na = species that produced flat responses of F_v/F_m to temperature in the frost experiment.

Species		FT_{50}			LT_{50}		
		High	Low	T_{span}	High	Low	T_{span}
Temperate							
E. lucida		50.2 (0.9) ab	−12.4 (1.1) ab	62.6 (0.3)	48.4 (0.2) b	−9.8 (0.1) c	58.2 (0.1)
N. cunninghamii		44.6 (1.0) d	−14.2 (1.5) b	58.8 (1.5)	47.7 (1.7) b	−9.9 (0.5) c	57.7 (2.2)
T. laurina		47.4 (0.9) bcd	−8.1 (1.6) ab	55.5 (2.5)	50.5 (1.4) ab	−7.0 (0.3) bc	57.5 (1.7)
A. smithii		46.8 (0.7) cd	−13.9 (1.1) ab	60.7 (1.5)	49.4 (0.4) b	−6.7 (1.2) bc	56.2 (0.8)
Tropical							
S. woollsii		50.7 (0.7) a	−6.9 (0.5) a	57.7 (0.5)	51.3 (0.3) ab	−4.4 (0.9) ab	55.7 (1.2)
H. trifoliolata		51.0 (0.8) a	−11.9 (0.2) ab	62.9 (0.6)	51.3 (0.1) ab	−5.4 (0.1) ab	56.6 (0.3)
C. australe		51.9 (0.7) a	Na	Na	54.6 (0.3) a	−5.7 (0.2) b	60.4 (0.5)
A. scholaris		49.3 (1.3) ab	Na	Na	54.5 (1.0) a	−1.7 (0.7) a	56.2 (1.7)
Species	*F*	13.8	6.47	3.68	9.01	16.6	1.40
	P	< 0.001	0.03	0.09	< 0.001	< 0.001	0.33
Run	*F*	0.36	0.64	0.08	1.57	0.01	0.81
	P	0.57	0.46	0.79	0.25	0.92	0.40

of the winter photoinhibition, *Castanospermum australe* had only a 24% reduction in initial F_v/F_m when exposed to the lowest temperature of –18 °C and *Alstonia scholaris* had random increases and decreases in initial F_v/F_m of less than 10% across the temperature range. There was no relationship between the low temperature causing a 50% reduction in initial F_v/F_m and the minimum temperature of the coldest month among the remaining species (Figure 2B). In contrast, the low temperature causing 50% damage to leaves was closely related to the minimum temperature of the coldest month in the species' native habitat (Figure 2C).

The T_{span} over which > 50% of initial F_v/F_m was maintained ranged between 56 and 63 °C (Table 3). Although T_{span} values could not be determined for the two tropical species, there was considerable overlap among the other species (F = 3.68, P = 0.09). There was even less variation in the T_{span} over which leaves were less than 50% damaged, with values ranging from 56 to 60 °C.

The temperatures causing a 50% reduction in initial F_v/F_m differed from the temperatures causing 50% damage to the leaf (Figure 3). During the heat treatment, there was a tendency for chlorophyll fluorescence to underestimate the temperature that caused 50% leaf damage. In contrast, chlorophyll fluorescence of all species overestimated the freezing temperature that caused 50% leaf damage.

Discussion

Leaves of the tropical rain forest species tended to have a greater heat tolerance (51–55 °C) than the temperate rain forest species (48–51 °C). These values for leaf tissue heat tolerance are similar to those reported previously for woody plants: temperate (38–52 °C), Mediterranean (48–55 °C) and tropical species (45–60 °C; Kappen 1981, Larcher 2000). The greater heat tolerance of the tropical species is consistent with the higher mean maximum temperatures of the tropical climate

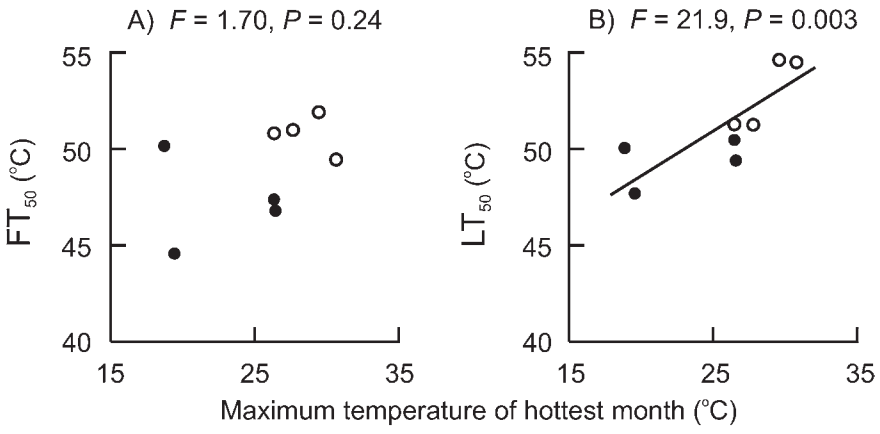

Figure 1. Relationships between maximum temperature of the hottest month (MTHM) in a species' native habitat and (A) the temperature causing a 50% reduction in initial F_v/F_m (FT_{50}) and (B) the temperature causing 50% damage to leaves (LT_{50}). Values represent individual species and are separated into temperate (●) and tropical (○) species. The results of linear regressions are given. The equation for leaf damage is LT_{50} = 0.53 MTHM + 37.47.

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Table 4. Reduction in chlorophyll fluorescence (F_v/F_m) of untreated leaves between April (early autumn) and July (midwinter). Values are means of six plants with standard errors in brackets. Results of one-way ANOVAs comparing values for species between seasons are given.

Species	F_v/F_m		F	P	% reduction in F_v/F_m
	April	July			
Temperate					
E. lucida	801 (8)	785 (6)	7.20	0.04	2.0
N. cunninghamii	820 (5)	783 (9)	14.6	0.01	4.5
T. laurina	829 (3)	755 (13)	27.0	0.003	8.9
A. smithii	806 (7)	691 (6)	127	< 0.001	14.3
Tropical					
S. woollsii	792 (3)	648 (10)	248	< 0.001	18.2
H. trifoliolata	791 (5)	676 (8)	106	< 0.001	14.5
C. australe	754 (6)	560 (20)	152	< 0.001	25.7
A. scholaris	817 (1)	116 (18)	1370	< 0.001	85.8

compared with the temperate climate (Table 2). For all species, the heat tolerance of photosynthesis and leaf tissue was at least 18 °C higher than the mean maximum temperature of the hottest month in the species' native habitat (cf. Tables 2 and 3). However, air temperatures can reach about 40 °C during extreme years in the species' native habitats. Furthermore, leaf temperatures can exceed air temperatures by as much as 10 °C (Hamerlynck and Knapp 1994, Larcher 2000). Therefore, rain forest species are able to avoid heat damage during average years and to prevent all but minimal damage during extreme events in their native habitats.

In general, the tropical species had a greater heat tolerance of photosynthesis than the temperate rain forest species (Table 3). The exceptions to this trend were the species from the warmest (*Alstonia scholaris*) and the coldest climates (*Eucryphia lucida*), which both underwent a 50% reduction in chlorophyll fluorescence (F_v/F_m) at 50 °C. Previous studies have shown that warm-climate species have a higher heat tolerance of photosynthesis than cool-climate species (Smillie and Nott 1979, Knight and Ackerly 2002, Salvucci and Crafts-Brandner 2004); however, in these studies, plants were grown under the contrasting conditions of their native habitat, and thus the finding takes no account of the capacity of these species for photosynthetic acclimation to the prevailing temperature (Monson and Williams 1982, Braun et al. 2002). When Knight and Ackerly (2002) grew the same species in a common environment, there was little difference in the heat tolerance of photosynthesis among coastal and desert species.

Foliar frost tolerance was higher in temperate species than in tropical species (Table 2), and had a close relationship with the minimum temperatures of the coldest month in the species' native habitat (Figure 2C). Many studies of frost tolerance in trees have shown that temperate species are more tolerant of chilling and freezing than tropical species (Sakai and Larcher 1987). The foliar frost tolerance of other Australian rain forest trees is consistent with their latitudinal distribution (Read and Hill 1989, Read and Hope 1989), but is not always consistent with the altitudinal distribution of species within a region (Read and Hill 1988). This inconsistency suggests that, in addition to foliar frost tolerance, frost tolerance in other tissues (e.g., buds and stems), annual productivity, reproductive physiology and regeneration niche (e.g., size of canopy gap or site exposure) are important in determining the altitudinal limits of tree species.

In contrast to the trends in foliar frost tolerance, there was no relationship between the frost tolerance of photosynthesis found for the rain forest species and winter temperature in the native habitat (Figure 2). This relationship was greatly weakened by the absence of the two tropical species from the warmest climates in this regression—their chronic photoinhibition in winter temperatures made it impossible to determine the frost tolerance of photosynthesis. In addition, the hardening temperatures would not have been low enough to reveal the full potential frost tolerance of the temperate species. However, the significant photoinhibition in the tropical species during winter (Table 4), when minimum temperatures were a moderate 5–11 °C, demonstrates the low frost tolerance of photosynthesis in the tropical species (Figure 2A). Cold-induced photoinhibition is commonly found in subtropical and tropical crops at temperatures between 0 and 10 °C (Baker et al. 1988). Furthermore, a similar trend was found when Mediterranean evergreen woody plants were grown in a common environment, with southern species having greater photoinhibition than more widespread species and northern species (Larcher 2000). Cold-induced photoinhibition has been found to limit the regeneration of species at the tree line (Ball et al. 1991) and may limit the latitudinal distribution of species.

The temperate and tropical species maintained > 50% of their initial chlorophyll fluorescence over a similar span of temperatures (Table 3). Previous work with the same rain forest species found that the temperate species maintained near maximum net photosynthetic rates over a wider range of both growth and acclimation temperatures than did tropical species (Cunningham and Read 2002, Cunningham and Read 2003*b*). These trends seem inconsistent, but they involve different aspects of photosynthesis. Maximum photosynthetic rates are likely to be related to daytime temperatures during the growing season. The ability of the temperate species to maintain

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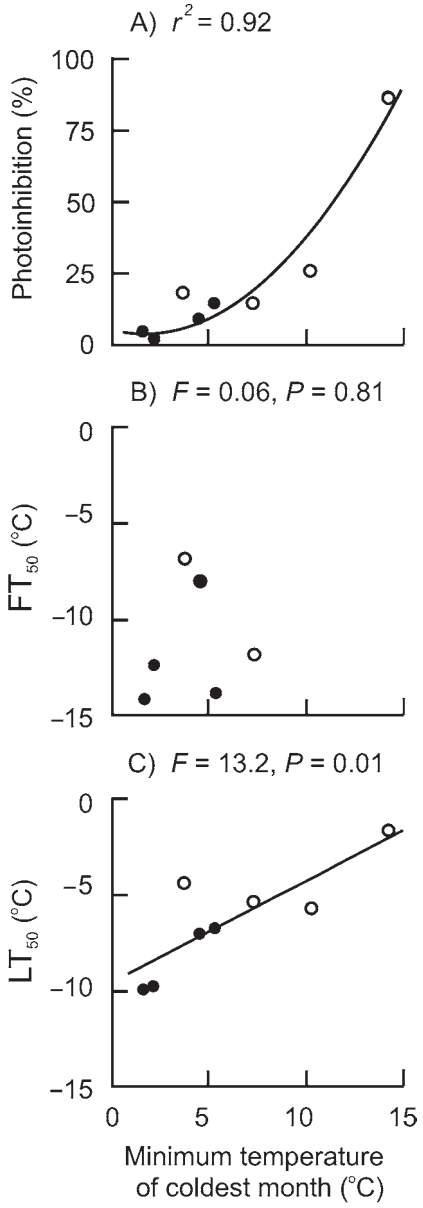

Figure 2. Relationships between minimum temperature of the coldest month (MTCM) in a species' native habitat and (A) winter photoinhibition (percentage reduction in autumn F_v/F_m); (B) the temperature causing a 50% reduction in initial F_v/F_m (FT_{50}); and (C) the temperature causing 50% visible leaf damage (LT_{50}). Values represent individual species and are separated into temperate (●) and tropical (○) species. The results of nonlinear (A) and linear regressions (B, C) are given. The equation for winter photoinhibition is $0.48MTCM^2 - 1.63MTCM + 4.99$ and the equation for leaf damage is $LT_{50} = 0.52MTCM - 9.54$.

maximum photosynthetic rates over a larger range of temperatures than the tropical species is likely to be an adaptation to the larger seasonal fluctuations in maximum temperature of the temperate zone than of the tropics. In contrast to photosynthetic rate, chlorophyll fluorescence measures the functional integrity of the photosynthetic apparatus. In evergreen species, such as those investigated in the present study, the photosynthetic apparatus is likely to be adapted to remain functional over the seasonal range of daytime temperatures in the plant's native habitat. Similarly, leaf tissue is likely to be adapted to resist damage over the full range between the temperature maximum and minimum in the species' native habitat. Although the native habitats of Australian temperate and tropical rain forest species differ in maximum and minimum temperatures, they have similar annual temperature ranges (Table 2). Therefore, maintenance of the photosynthetic apparatus, as well as avoidance of leaf damage, over a similar T_{span} among temperate and tropical rain forest species is consistent with the similar annual temperature ranges of their climates.

The temperature tolerances of photosynthesis and leaf tissue differed for most species (Figure 3). During the heat treatment, most species showed a 50% reduction in chlorophyll fluorescence at a lower temperature than that causing 50% damage to the leaf. Similarly, *Picea glauca* (Moench) Voss showed a 50% reduction in chlorophyll fluorescence at 45–46 °C, but did not show 50% needle damage until 49 °C (Bigras 2000). The lower threshold for damage to the photosynthetic apparatus compared with that for visible leaf damage suggests that photoinactivation of photosystem II or reversible damage to the thylakoid membrane occurs at lower temperatures than those causing permanent damage to the cell membrane (Levitt 1980, Anderson et al. 1997). In contrast, the threshold temperature for low temperature damage to the photosynthetic apparatus was consistently higher than that causing visible leaf damage. This discrepancy suggests that short-term recovery of the photosynthetic apparatus can occur after freezing temperatures that will lead to cell death within several days. A study of *Rhododendron ferrugineum* L. showed that frost tolerance estimated from chlorophyll fluorescence measurements increased by more than 10 °C during the first four days following the treatment (Neuner and Buchner 1999). However, frost tolerance of *Rhododendron ferrugineum* estimated from chlorophyll fluorescence measured a day after treatment was lower than that based on the visual assessment of leaf damage after one week, which is the opposite of our findings. This indicates that the timing of measurements may be an important factor accounting for discrepancies among measures of temperature tolerance, and that the effect of timing is species dependent.

Chlorophyll fluorescence measurement is widely considered a reliable method for rapidly estimating relative temperature tolerance among taxa because of its close correlation with visible leaf damage (e.g., Lindgren and Hällgren 1993, Binder and Fielder 1996). However, we found that chlorophyll fluorescence and assessment of visible leaf damage gave different rankings for the temperature tolerance of rain forest species (Table 3). For example, visual assessment of leaf damage predicted that, among the species from the warmest habitats, *Alstonia scholaris* has one of the highest heat tolerances and, among the species from the coldest habitats, *Eucryphia lucida* has one of the lowest heat tolerances, whereas based on chlorophyll fluorescence, one would predict that both species have intermediate heat tolerances.

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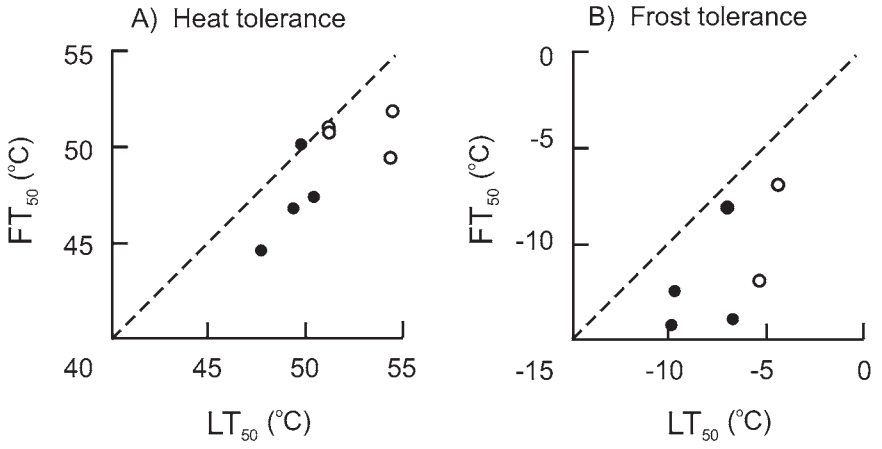

Figure 3. Relationships between the temperature causing a 50% reduction in initial F_v/F_m (FT_{50}) and the temperature causing 50% damage to leaves (LT_{50}) estimated during (A) the heat tolerance experiment and (B) the frost tolerance experiment. Values represent individual species and are separated into temperate (●) and tropical (○) species. The dashed line delimits a 1:1 relationship.

Discrepancies between the measures of extreme temperature tolerance are likely a result of the different processes measured, and differences in the scale and timing of measurements. Chlorophyll fluorescence measures the (potentially reversible) photoinactivation of photosystem II over a small proportion of the leaf within 2 h of the treatment, whereas visual assessment measures permanent damage to the whole leaf 10 days after the treatment. Our results suggest that chlorophyll fluorescence measurements made within hours of the treatment cannot, in isolation, predict the relative temperature tolerance of a species, and they emphasize the importance of making visual assessments of tissue damage to determine tolerance to extremes of temperature. Measurements of chlorophyll fluorescence made at the same time as visual assessments (i.e., 10 days after the treatment) may predict similar temperate tolerances and are recommended for future studies.

In conclusion, the temperature tolerances of the rain forest tree species determined by visual assessment of leaf damage were strongly correlated with the temperature regime in the native habitat. Leaves of tropical species had a higher heat tolerance and a lower frost tolerance than leaves of the temperate species. In contrast, temperature tolerance of photosynthesis did not necessarily correlate with the climate in a species' native habitat. All species had a similar T_{span} over which the photosynthetic apparatus was maintained and tissue damage was avoided, reflecting the similar annual temperature range of the climate in their native habitats. The temperature tolerances of all the species studied are likely to be adequate to avoid damage to the photosynthetic apparatus and leaf tissue during average years, and to minimize damage during extreme events, in the natural habitat. This suggests that there is strong selection for tolerance of extreme temperature events in Australian rain forest tree species. However, the successful cultivation of many temperate and tropical rain forest trees well outside their native habitats emphasises the importance of productivity at moderate temperatures in determining competitive outcomes and the distributional limits of rain forest species.

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