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Invasive species can handle higher leaf temperature under water stress than Mediterranean natives

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ABSTRACT

Thermal tolerance of Photosystem II (PSII) highly influences plant distribution worldwide because it allows for photosynthesis during periods of high temperatures and water stress, which are common in most terrestrial ecosystems and particularly in dry and semi-arid ones. However, there is a lack of information about how this tolerance influences invasiveness of exotic species in ecosystems with seasonal drought. To address this question for Mediterranean-type ecosystems (MTE) of the Iberian Peninsula, we carried out an experiment with fifteen phylogenetically related species (8 invasive and 7 native, *Pinus pinaster* Ait., *Pinus radiata* D. Don, *Schinus molle* Linn., *Elaeagnus angustifolia* L., *Eucalyptus globulus* Labill., *Acacia melanoxylon* R. Br., *Gleditsia triacanthos* L., *Pistacia terebinthus* L., *Rhamnus alaternus* L., *Anagyris foetida* L., *Colutea arborescens* L., *Oenothera biennis* L., *Epilobium hirsutum* L., *Achillea filipendulina* Lam. and *Achillea millefolium* L.). Seedlings were grown and maximal photochemical efficiency of PSII (Fv/Fm) was measured at two water availabilities (well-watered and with water stress). PSII thermal tolerance measurements were related to specific leaf area (SLA), which varied significantly across the study species, and to the mean potential evapotranspiration (PET) of the month with the lowest precipitation in the native areas of both groups and in the invaded area of the Iberian Peninsula. Additionally, PSII thermal tolerance measurements under water stress were phylogenetically explored. Invasive and native species neither differed in SLA nor in their thermal tolerance under well-watered conditions. For well-watered plants, SLA was significantly and positively related to PSII thermal tolerance when all species were explored together regardless of their invasive nature. However, this relationship did not persist under water stress and invasive species had higher plastic responses than Mediterranean natives resulting in higher leaf temperatures. Higher PSII thermal tolerance could explain invasiveness because it allows for longer periods of carbon acquisition under water stress. In fact, PSII thermal tolerance was positively related to the PET of the invaded and native areas of the Iberian Peninsula. PSII thermal tolerance was not related to PET at the native range of the invasive species, suggesting that successful invasive species were plastic enough to cope with novel dry conditions of the Iberian Peninsula. Moreover, our phylogenetic results indicate that future scenarios of increased aridity in MTE associated to climate change will filter invasion success by taxonomic identity. This study reveals the importance of studying ecophysiological traits to understand and better predict future biological invasions.

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1. Introduction

Temperature and water availability are the most important abiotic filters controlling plant distribution including the spread of invasive species (Hamilton et al., 2005; Hawkins et al., 2003; Thuiller et al., 2005). In Mediterranean-type ecosystems (MTE), high temperatures limit physiological plant activity, especially during summer drought (Castro-Diez and Montserrat-Martí, 1998;

Cowling et al., 2005; Mitrakos, 1980; Sánchez-Gómez et al., 2006). Specifically, high temperature damage photochemical and dark reactions of photosynthesis, being Photosystem II (PSII) the photosynthetic component most susceptible to suffer an irreversible damage (Baker, 1991; Havaux, 1993; Qiu and Lu, 2003). Damages in PSII can significantly affect carbon acquisition at short and long term reducing plant competition and survival (Berry and Björkman, 1980; Knight and Ackerly, 2003; Reich and Oleksyn, 2004). Therefore under a competition scenario, our hypothesis is that invasiveness in MTE may be associated to possess or develop a higher photosynthetic tolerance to elevated temperatures and drought in comparison with native species.

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PSII thermal tolerance is one decisive component of heat resistance (Salvucci and Crafts-Brandner, 2004). It increases by exposition to moderately elevated temperatures (Havaux, 1993; Krause et al., 2010) and drought (Ghouil et al., 2003; Havaux, 1992; Ladjal et al., 2000; Valladares and Pearcy, 1997). Physiological and biochemical processes such as stabilization of membranes by osmotically active solutes like soluble sugars and glycine–betaine (Hüve et al., 2006), and changes in thylakoid membrane composition including the accumulation of zeaxanthin (Havaux and Tardy, 1996) play an important role in plant responses to stability of photosynthetic electron transport when heat stress is related to drought. For instance, Knight and Ackerly (2001, 2003) showed that species adapted to heat and water stress conditions has the capacity to express high levels of low molecular weight heat shock proteins (sHsp), which protect the protein complex of the chloroplast electron transport chain against denaturalizing heat.

Moreover, two morphologically contrasted strategies can also contribute to avoid the effect of high temperature. Plants can augment transpiration to reduce leaf temperature (Niinemets and Valladares, 2006) or reduce leaf size and position leaf blade parallel to the incident radiation to decrease the amount of photons reaching the leaf blade (Pastenes et al., 2005; Xu et al., 2007). On average, when water availability can support transpiration demand, plants do not reduce the specific leaf area (SLA, projected leaf area per unit leaf dry mass) and water availability is positively related to growth temperature (Loveys et al., 2002) with higher leaf size and SLA being more common at wetter sites (Wright et al., 2006). But, large leaves have higher potential evaporative demand compared to small size leaves under similar incident radiation due to the higher thickness of their boundary-layer, which reduces energy and gas exchange (Baldocchi and Wilson, 2001). Hence, reduction of leaf size in dry environments has been explained on the basis of leaf boundary-layer conductance for heat and gaseous transport (Niinemets et al., 2007), and seems to be an important driver of adaptation to constantly water stressed environments such as deserts (Knight and Ackerly, 2003).

Arguments supporting SLA reduction to cope with high temperatures in water stressed environments may impose a conflict to potentially invasive plants because they typically exhibit elevated SLA and total leaf areas (Daehler, 2003; Hamilton et al., 2005; van Kleunen et al., 2010). This conflict is even more patent for the invasive species of the Iberian Peninsula invaders because most of them grow and flower during summer, the period when drought occurs (Godoy et al., 2009). However, in MTE of the Iberian Peninsula high temperatures and low precipitation do not occur along the whole year (Oliveira and Peñuelas, 2004; Valladares et al., 2002). Therefore, we hypothesize that this temporal combination of both abiotic filters is not long and strong enough to drive a reduction of SLA in alien species (Knight and Ackerly, 2003), and that their invasiveness is associated to high levels of PSII thermal tolerance. For well-watered plants, we expect invasive species to exhibit higher levels of PSII thermal tolerance than native species, particularly if they have leaves with higher SLA due to the reduced leaf boundary-layer conductance that is expected for them (Niinemets et al., 2007).

However, under water stress, alien species should show higher PSII thermal tolerance levels as a feature providing invasiveness and the positive relationship between PSII thermal tolerance and SLA may not persist due to the existence of complex interactions among specific biochemical and physiological stress responses (Havaux and Tardy, 1996; Hüve et al., 2006). Additionally, we hypothesize that if PSII thermal tolerance under drought is an adaptive key trait to invade MTE, it should be highly associated to mean potential evapotranspiration (PET) of the driest month (as a proxy for water stress) within the range distribution of invasive species in the Iberian Peninsula. Finally, we will explore whether PSII thermal tolerance under water stress is phylogenetically clustered, i.e., whether some taxonomic families are better pre-adapted to cope with the future increase of aridity predicted for MTE of the Iberian Peninsula (Christensen et al., 2007; Gu et al., 2008; Ogaya and Peñuelas, 2004).

In order to test these predictions, we determine the thermal stability of PSII by chlorophyll fluorescence measurements in leaves of fifteen plant species (8 invasive and 7 native species) growing under control and water stress environments. Data of critical temperatures reducing 15 and 50% of maximal photochemical efficiency (Fv/Fm values), were determined together with SLA under both water treatments.

2. Material and methods

2.1. Study species

Our species sets comprised alien species that are clearly invasive in the Iberian Peninsula, local dominants and with potential impact in the invaded ecosystems (Pyšek et al., 2004; Richardson et al., 2000; Sanz Elorza et al., 2004; Valéry et al., 2008). Invasive species selected aimed to represent contrasting life forms with broad variation in leaf area of exotic species invading the Iberian Peninsula. Thus, we selected one conifers tree, two legume trees, three broadleaves trees, and two herbaceous species (Table 1). Each invasive species was paired with their nearest taxonomic native counterpart in the Iberian Peninsula to avoid phylogenetic effects between invasive–native comparison (Felsenstein, 1985) (Table 1). During spring and summer of 2007, seeds of these species were germinated on a 3:1 peat/sand substrate and later seedlings were transplanted to 0.5 l pots with the same substrate. Plant individuals were cultivated in a greenhouse, which removed the 30% of full sun radiation; they were twice fertilized with 1.2 g NPK (NITROFOSKA 12-12-17) and maintained well irrigated. Daily PPFD was $32 \text{ mol m}^{-2} \text{ day}^{-1}$ and the mean \pm SE of the maximum, minimum and mean temperature during the experimental period were 31.9 ± 0.5 °C, 14.2 ± 0.3 °C and 20.6 ± 0.3 °C, respectively.

2.2. Water stress and thermal tolerance measurements

After 25–40 days of growth, water stress was slowly imposed during 7–8 days and then relative water content (RWC) of leaves was determined. In comparison to the watered control, water stress

Table 1
Study species included in the thermal tolerance experiment.

Family	Invasive species	Family	Native species
Pinaceae	<i>Pinus radiata</i> D. Don.	Pinaceae	<i>Pinus pinaster</i> Ait.
Fabaceae	<i>Acacia melanoxylon</i> R. Br.	Fabaceae	<i>Anagyris foetida</i> L.
Fabaceae	<i>Gleditsia triacanthos</i> L.	Fabaceae	<i>Colutea arborescens</i> L.
Anacardiaceae	<i>Schinus molle</i> Linn.	Anacardiaceae	<i>Pistacia terebinthus</i> L.
Elaeagnaceae	<i>Elaeagnus angustifolia</i> L.	Rhamnaceae	<i>Rhamnus alaternus</i> L.
Asteraceae	<i>Achillea filipendulina</i> Lam.	Asteraceae	<i>Achillea millefolium</i> L.
Onagraceae	<i>Oenothera biennis</i> L.	Onagraceae	<i>Epilobium hirsutum</i> L.
Myrtaceae	<i>Eucalyptus globulus</i> Labill.		

ranged RWC values from 3.0 to 3.5% reduction on *Pinus* species to 20–28% RWC reduction on *Elaeagnus angustifolia*, *Achillea millefolium*, *Eucalyptus globulus* and *Anagyris foetida*. The remaining species presented intermediate reductions on RWC. The tolerance to high temperatures, related to the thermostability of PSII photochemistry, was evaluated by non-destructive measurements of chlorophyll fluorescence determining the maximal efficiency of PSII photochemistry (Fv/Fm) using a FMS2 fluorometer (Hansatech, UK). Decrease in the Fv/Fm ratio under high temperature stress have been thought to reflect damage to the photosynthetic apparatus (Ghouil et al., 2003; Havaux, 1992; Krause and Weis, 1991). For each species, six plants from control and six plants from the water stress treatment were first acclimated at low light levels ($<5 \mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD) and then used to evaluate the effect of increasing temperature on Fv/Fm values. From these individual selected, two similar sized pieces of mature and healthy leaves were cut and placed on moist filter paper and recovered with a plastic film and were put over a thermal controlled plate. Over there, we put an aluminium black plate with holes adjusted in order to expose the leaves for fluorescence measurements with the fluorimeter fiber optic at 60° . The temperature values registered on leaves were obtained with a thermocouple installed just a side of them and registered with a DT-610B thermocouple thermometer (CEM, Germany). The utilized apparatus, developed by OCHE Control y Equipamientos (Zaragoza, Spain), includes a Syros temperature digital control unit (DITEL, Barcelona, Spain), connected to a plate with resistances below and a peltier cooler, which permitted a controlled linear increase in temperature of $0.5^\circ\text{C min}^{-1}$. The initial fluorescence measurement was taken around $25\text{--}30^\circ\text{C}$, and after an increase of approximate 2°C , new Fv/Fm values were obtained until the highest temperature at which no fluorescence signal was observed. The responses of Fv/Fm with increasing temperature were analyzed by a sigmoidal fit model using the data obtained for each leaf piece. In each fitted curve, we determine the critical temperature, which promotes reduction in 15% and 50% of initial Fv/Fm values (T_{15} , T_{50}) (Fig. 1).

Finally, six randomly selected, mature and healthy leaves of each species were collected from different individuals and its image was captured in a scanner (Lexmark X1130) with a resolution of 150DPI. After this, leaves were dried during 3 days at 60°C and weighed with or without petiole. UTHSCSA Image tool 2.00alfa was used to measure the projected leaf area and then SLA was calculated based on dry mass weight of whole leaf without petiole.

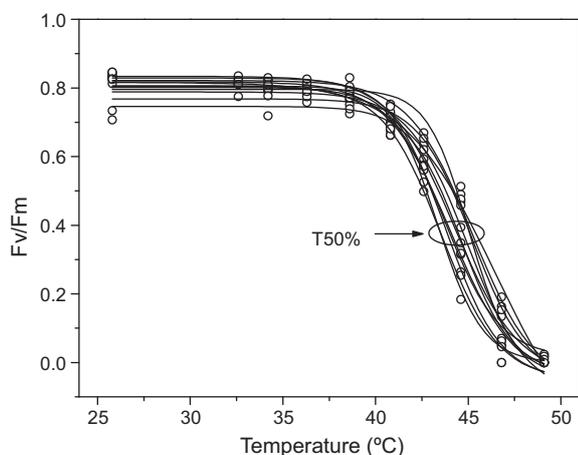


Fig. 1. Example of sigmoidal adjusted curves of Fv/Fm values in relation to increasing temperature for *Acacia melanoxylon* leaves of watered plants as used to determine the critical temperature T_{50} (temperature at which Fv/Fm is reduced by 50%; same rationale used for T_{15}).

2.3. Climatic data

We extracted maximum, minimum and mean monthly world temperature, and monthly world precipitation at a spatial resolution of 30 arc-seconds from the WorldClim database (Hijmans et al., 2009, <http://www.worldclim.org/>) to calculate potential evapotranspiration (PET) following Hargreaves and Samani (1985) and Samani (2000) methods. Climatic variables were overlaid with distribution maps of the species and geometrically corrected in ArcGIS 9.3 to obtain monthly PET through the distribution range of the species. Distribution maps of every invasive and native species in the Iberian Peninsula were obtained from the Atlas of invasive plants in the Iberian Peninsula (Sanz Elorza et al., 2004) and Anthos Project (<http://www.anthos.es/>, last access 7 January 2010) respectively, whereas distribution maps of invasive species in their native range were obtained from the Global Biodiversity Database (<http://data.gbif.org/>, last access 7 January 2010). After exploration of the climatic data we decided to select July's PET values for the Iberian Peninsula for further analysis because it is, on average, the month with the highest PET and lowest precipitation. In the case of the native range of the exotic species, July's PET values were also selected for all species except for those of the Southern hemisphere (*S. Molle*, driest month September, and *A. melanoxylon* and *E. globulus* driest month January).

2.4. Statistical analysis

Prior to statistical analyses normality was checked and only SLA data was log transformed to reach the normality assumptions. Linear regressions were performed to evaluate the relationship between SLA and T_{50} for well-watered and water stress treatments, SLA and PET, and finally for every single species, T_{50} under water stress treatment and PET in the Iberian Peninsula and in the region of origin of invasive species. Also within invasive species, a pairwise *t*-test was conducted to verify whether exist differences in PET between the invaded range in the Iberian Peninsula and in their native range.

To assess differences in PSII thermal tolerance between invasive and native species, two factorial-ANOVAs were performed. T_{15} and T_{50} were the dependent variables and status (invasive vs. native) and stress (with and without water stress) the categorical factors. In addition, a two-way ANOVA was performed to evaluate differences in PET and SLA between invasive and native species. Before performing these analyses of variance, a multiple regression was conducted to evaluate if the taxonomic pairs selected had an influence on our results. Dependent variables were the differences in each measured trait variable within pairs (T_{15} , T_{50} , SLA) and the independent was the phylogenetic distance within pairs. The phylogenetic distance from one species to another between all the species pairs was calculated through the first common ancestor to both species using the plant phylogenetic supertree described by Soltis et al. (2000) and modifications by Bremer et al. (2003).

Phylogenetic conservatism of T_{50} under water stress was also explored. First, we built a phylogenetic tree with the study species as terminal tips using the maximally resolved seed plant tree available in Phylomatic (<http://www.phylodiversity.net/phylomatic/>). Branch tree lengths were assigned using the clade age calibrated in million of years and estimated by Wikstrom et al. (2001). Later, we implemented T_{50} data and the phylogenetic noted-data tree into the Analysis of Traits module of Phylocom (AOT) (<http://www.phylodiversity.net/phylocom/>). AOT calculates divergence-convergence degree at each internal node of the tree. The standard deviation between trait means of daughter nodes was used as a proxy of the degree of divergence at the focal node (i.e., divergence size). Significance of divergence size at 5% was esti-

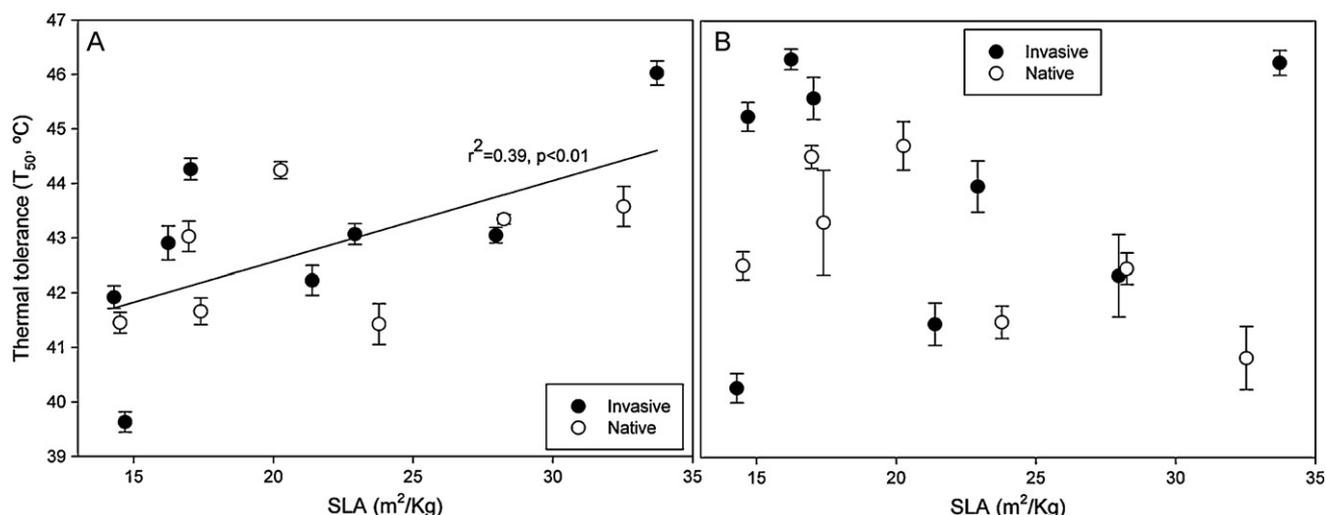


Fig. 2. Relationship between PSII thermal tolerance and SLA. (A) Well-watered, (b) water stressed plants. Water stress blur the positive relationship between thermal tolerance and SLA ($r^2 = 0.04$, $p = 0.59$).

ated by randomly permuting trait values (10,000 permutations) across the tips of the phylogeny.

3. Results

According to our expectations, we observed a positive relationship between SLA and T_{50} in well-watered plants (Fig. 2A) but this association vanished under drought ($r^2 = 0.04$, $p = 0.79$) (Fig. 2B), indicating that differential PSII thermal tolerance responses to water stress were not related to their morphological characteristics. Besides, PET and T_{50} under water stress were positive related in the Iberian Peninsula for both groups but not for PET in the native range of invasive species ($r^2 = 0.10$, $p = 0.45$) (Fig. 3A). There was no relationship between PET and SLA ($r^2 = 0.02$, $p = 0.98$) (Fig. 3B). Finally, PET values significantly differed between the Iberian Peninsula and the native range of invasive species (Iberian Peninsula = 224.22 ± 4.01 , native range = 201.32 ± 7.67 , $t_{1,7} = 2.87$, $p = 0.025$).

Invasive and native species showed similar levels of T_{15} for well-watered and water stressed plants but an interaction occurred for T_{50} values (Table 2). While both groups showed similar values of T_{50} for well-watered plants, invasive species showed higher PSII thermal tolerance when plants were water stressed (Fig. 4). These differences were not due to higher SLA or PET (SLA, Invasive = 20.45 ± 2.17 Native = 22.39 ± 2.90 ; PET, Invasive = 224.22 ± 3.99 Native = 220.85 ± 4.43) as the two-way ANOVA demonstrated no differences between both groups (Table 2). Moreover, all these analyses of variance were not influenced by pairwise invasive–native selection since multiple regression was not significant ($F_{1,43} = 1.39$, $p = 0.25$).

Table 2

Factorial-ANOVA crossing status (invasive vs. native) with water stress for T_{15} and T_{50} mean values \pm SE are shown in Fig. 4. Additionally, F and p values of two-way ANOVA for the differences in ETP and SLA between invasive and native species are also given.

	T_{15}		T_{50}		ETP		SLA	
	F	p	F	p	F	p	F	p
Stress	23.67	<0.001	53.81	<0.001	NA	NA	NA	NA
Status	0.79	0.37	16.69	<0.001	0.08	0.78	0.06	0.83
Status \times Stress	0.81	0.36	4.07	0.04	NA	NA	NA	NA

NA, non applicable.

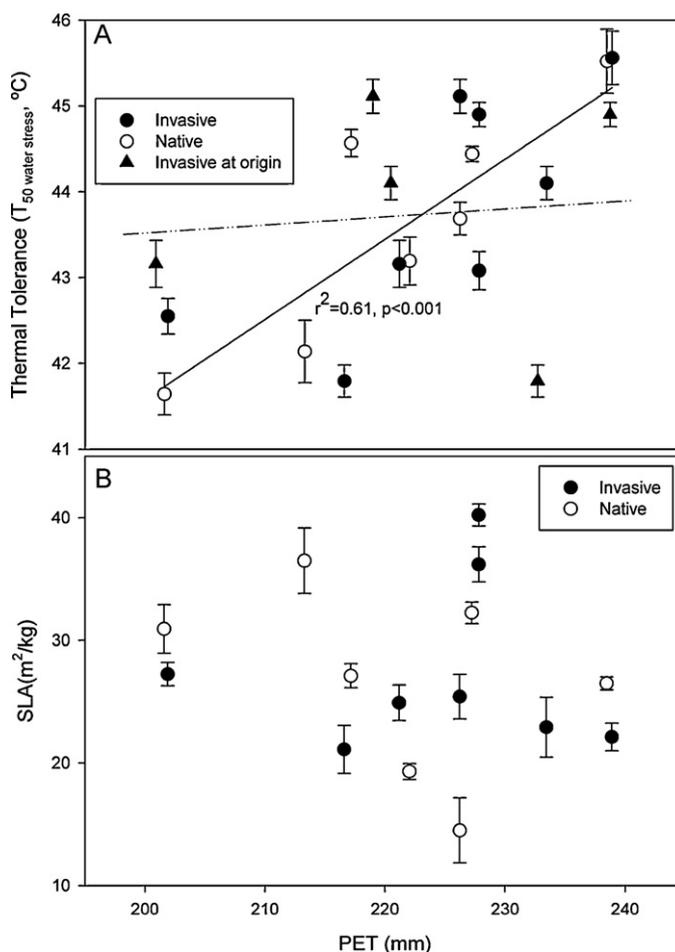


Fig. 3. Relationship between potential evapo-transpiration (PET) and PSII thermal tolerance (T_{50}) under water stress for invasive and native species in the Iberian Peninsula (circles, solid line) and at origin for invasive species (triangles, dash-dot line) (A), and between ETP and SLA (B) for both groups. PET values represented are those for the month with the highest mean values of the year, which in the Iberian Peninsula corresponds to July.

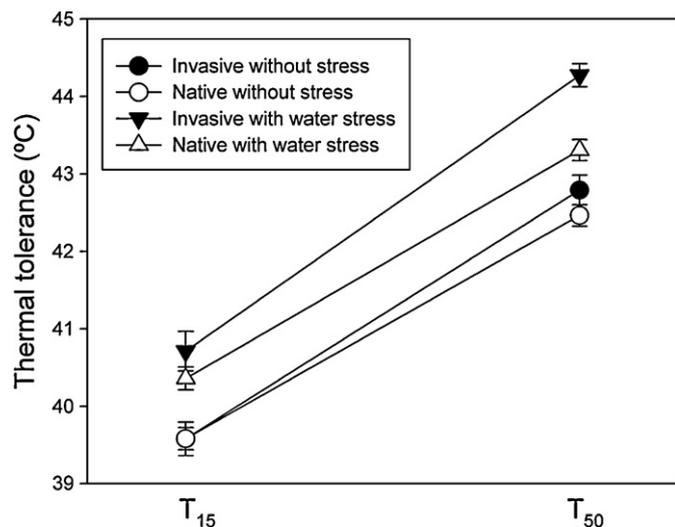


Fig. 4. Thermal tolerance (T_{15} and T_{50}) of invasive and native species. Circles represent watered plants, and triangles water stressed plants. Mean \pm SE is shown.

T_{50} under drought variance explained by the phylogenetic structure was significant ($r^2 = 0.36$ $p < 0.01$) (Fig. 5). The two Fabaceae nodes presented significant convergences between them and enough significant divergences to their daughter nodes. In addition, *Achillea* species presented the lowest T_{50} under drought, having significant divergences to their daughter node.

4. Discussion

PSII thermal tolerance of both invasive and native species was similar for well-watered plants. But under water stress, invasive

species were able to handle higher leaf temperature than Mediterranean natives (Fig. 4). In MTE, water is not only important for photosynthesis but also for controlling energy balance of leaves by plant transpiration. Thus, when water becomes scarce, exhibit elevated PSII thermal tolerances become crucial for species survival and competition because they maintain carbon acquisition (Stancato et al., 2001). Our results suggest that the higher T_{50} under drought of alien species was associated to their invasiveness because it was highly and positively related to PET values of the driest month in the invaded range of the Iberian Peninsula (Fig. 3A). Differences observed were not due to different climatic environmental conditions in the invaded range or morphological traits since PET in the Iberian Peninsula and SLA values did not differ between both groups (Table 2 and Fig. 2). Therefore, our results suggest that higher PSII thermal tolerance under water stress can be a trait associated with invasiveness in MTE and probably in other ecosystems with seasonal drought.

Many morphological traits such as high plant height and SLA, clonal growth, or low root: shoot ratio has been associated with invasiveness (Daehler, 2003; Pyšek and Richardson, 2007; van Kleunen et al., 2010). However, physiological traits, which are much poorly understood, can be as important as morphological traits explaining invasiveness, especially in stressful ecosystems. For instance, if we focus on SLA, previous literature proposes that a trade-off is imposed in invasive species between exhibiting higher SLA than natives to synthesize more carbon and being more competitive, and diminishing SLA to reduce heat stress and water loss. But with the ecophysiological data gathered here we suggest that this trade-off can be diminished if exotic species augment their PSII thermal tolerance rather than reduce their SLA as we reported here. Previous studies such as those by Niinemets (2001), Niinemets et al. (2007), and Wright et al. (2004) relating morphological leaf characteristics with ecophysiological traits can be very valuable to predict high levels of PSII thermal tolerance in certain invasive species, not

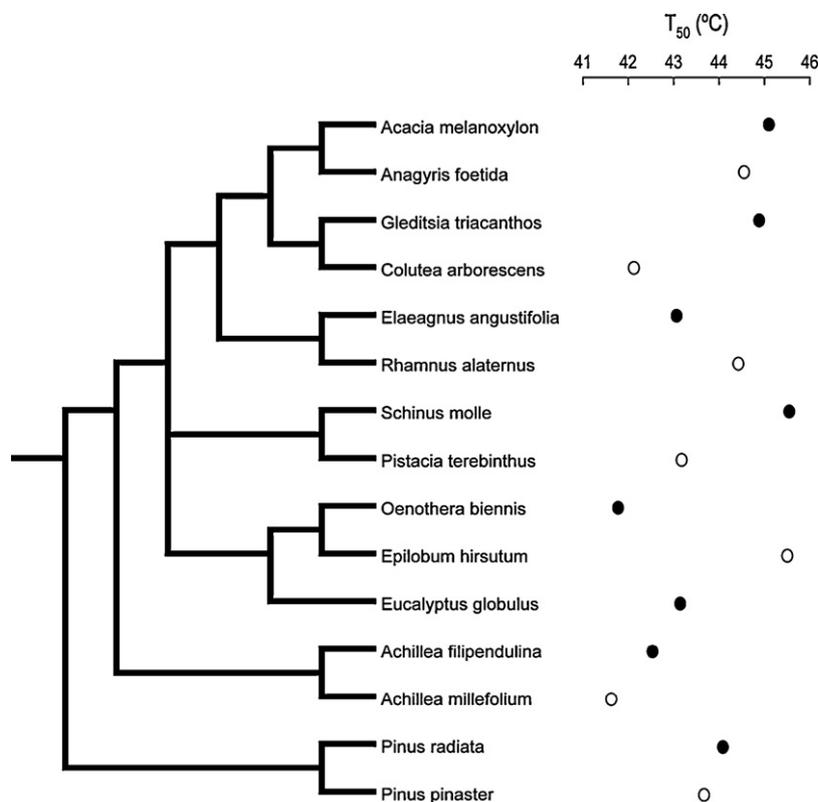


Fig. 5. Phylogenetic tree representing the thermal tolerance under water stress values across invasive and native species.

included in this study, which have important ecological and economical impacts worldwide. For example, we predict a high PSII thermal tolerance in invasive species with high photosynthetic and dark respiration rates, high leaf nitrogen and low leaf live span such as *Ulmus pumila*, *Ailanthus altissima* and *Datura stramonium* (Godoy et al., 2010, unpublished manuscript).

The climatic conditions in the native range of the plant species can be important to understand PSII thermal tolerance differences under drought between the two groups of species (Table 2, significant interaction status \times stress). If invasive species came from regions with more water deficit than the Iberian Peninsula, we can argue that a higher level of PSII thermal tolerance was selected in the region of origin and thus pre-adapted alien species to invade MTEs of the Iberian Peninsula. We should thus find a positive relation between T_{50} under drought and PET in their regions of origin as we found a positive relationship between T_{50} under drought and PET in the Iberian Peninsula. However, we found that invasive species came from regions with lower PET and, T_{50} under drought was not positively related to PET in the native range (Fig. 3A). These results have two possible explanations. Perhaps novel stressful climatic conditions have lead to an evolutionary process conferring invasiveness to alien species able to develop higher PSII thermal tolerance under water stress in comparison with natives, or, in contrast, differences between both groups could be the consequence of higher plastic responses of invasive species to novel stressful conditions of the Iberian Peninsula. Previous results have shown that PSII thermal tolerance is selected to be plastic rather context-specific because thermal stress is highly variable across space and time scales (Gimeno et al., 2009; Knight and Ackerly, 2003). Thus, plastic responses rather than evolutionary processes are likely to occur and the disagreement of the adaptive value of T_{50} under drought and PET in the region of origin of invasive species and in the invaded range of the Iberian Peninsula may be more related to differences in the severity of climatic conditions between regions. That is, only when water and heat stress is high enough, PSII thermal tolerance becomes adaptive.

We have found significant and positive relationship between PSII thermal tolerance and SLA for well-watered plants regardless of their invasive nature (Fig. 2) supporting the idea that seasonal combination of water and heat stress during summer in MTEs of the Iberian Peninsula is not strong enough to blur this positive relationship. Effects of leaf morphology on PSII thermal tolerance are expected because leaf temperature is higher in larger and regular leaves than in smaller or well lobed ones due to the low boundary layer conductance of the latter (Baldocchi and Wilson, 2001). Roth-Nebelsick (2001) modeling heat transference by small leaves under low wind speed demonstrated that higher heat dissipation efficiency occurred on smaller and more dissected ones. According to this, under zero or extremely low wind conditions, larger and less lobed leaves tends to present higher boundary layer thickness that will tend to reduce convective heat and moisture loss. For instance, an evolution to a reduction in SLA has been found in species that adapted over geological time to different climatic conditions from wet tropical to Mediterranean water stressed environments in the Mediterranean basin (Herrera, 1992).

Previous results have found a negative correlation between SLA and PSII thermal tolerance of species evolved in constantly dry areas (deserts) but they did not observe this negative relationship when plant were grown in a common garden experiment. Besides, species from deserts showed higher values of PSII thermal tolerance related to SLA when they were compared to those from milder areas (coastal environments) (Knight and Ackerly, 2003). They interpreted the lower SLA values of desert species as a convergent trait in plant lineages that evolved into thermally stressful environments with lower annual precipitation. However, in common garden experiments they could not observe these inter-

specific differences in relation to thermal tolerance and even the negative relationship with SLA. They supposed that these disagreements were due to differences in the stressful conditions in the field versus those in the glasshouse. Gathering these results with ours, we think that our study is an example of results dependency to gradient stress (Nicotra et al., 1997; Schlichting, 1989). Therefore, positive, negative or no relationship between PSII thermal tolerance and SLA may be influenced by to what extent the ecosystems studied are water and heat stressed for plant life, and by the evolutionary history of the species living and invading them.

Iberian Peninsula will suffer an increased aridity according to climate change predictions for MTE. Temperature rise, decreased rainfall, and increased frequency of extreme events such as heat waves will be the main factors driving this change (Christensen et al., 2007; Gu et al., 2008; Ogaya and Peñuelas, 2004). According to our results, invasive species will cope better with this new climatic scenario than natives because they possess higher PSII thermal tolerance values under drought and present also higher plastic responses to water variation. For instance, high plastic PSII thermal tolerance responses can be crucial to cope with unpredictable extreme heat waves. However, this general trend may have some exceptions and since PSII thermal tolerance under water stress had a phylogenetic pattern (Fig. 5), climatic change will filter invasion by taxonomic identity of alien species. While nitrogen-fixing species represented by Fabaceae family seems better pre-adapted to future Mediterranean aridity, Asteraceae family represented by *Achillea* species is positioned on the other end. Phylogenetic results for Fabaceae were expected because nitrogen-fixing species usually display a high nitrogen leaf concentration, which is associated with high levels of PSII thermal tolerance (Niinemets et al., 2007; Wright et al., 2004).

5. Conclusions

Our study demonstrates that invasive species can handle higher leaf temperature under water stress and can display higher plastic PSII thermal tolerance responses than Mediterranean natives. PSII thermal tolerance responses under water stress were adaptive to invasion in the Iberian Peninsula because it was related to the heat stress that plants suffer during the month with the lowest precipitation. On the contrary, the positive relationship between PSII thermal tolerance and SLA regardless of the invasive nature of the species for well-watered plants reflects that heat tolerance responses without drought are related to leaf morphological traits; it also suggests that summer drought in MTEs of the Iberian Peninsula is not strong enough to drive an evolutionary change of leaf area reduction to avoid water loss during heat stress.

Summarising, for a better understanding of biological invasions, especially in seasonally stressful ecosystems such as MTEs of the Iberian Peninsula, we have shown the importance of studying key physiological traits for plant competition and survival (e.g. PSII thermal tolerance). We should also have to understand how physiological and morphological traits are correlated under different environmental conditions, and finally, more research efforts are needed to show their adaptive value regarding the invasion of novel environments, which could be addressed by bottom-up processes where physiological traits are related to regional-scale variables such as range distribution. These analyses will provide valuable knowledge to better predict future spatial-temporal dynamics of biological invasions driven by climate change.

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References

- Baker, N.R., 1991. Possible role of photosystem II in environmental perturbations of photosynthesis. *Physiologia Plantarum* 81, 563–570.
- Baldocchi, D.D., Wilson, K.B., 2001. Modeling CO₂ and water vapor exchange of a temperate broadleaved forest across hourly to decadal time scales. *Ecological Modelling* 142, 155–184.
- Berry, J., Björkman, O., 1980. Photosynthetic response and adaptation to temperature in higher plants. *Annual Review of Plant Physiology* 31, 491–543.
- Bremer, B., Bremer, K., Chase, M.W., Reveal, J.L., Soltis, D.E., Soltis, P.S., Stevens, P.F., Anderberg, A.A., Fay, M.F., Goldblatt, P., Judd, W.S., Kallersjö, M., Karehed, J., Kron, K.A., Lundberg, J., Nickrent, D.L., Olmstead, R.G., Oxelman, B., Pires, J.C., Rodman, J.E., Rudall, P.J., Savolainen, V., Sytsma, K.J., van der Bank, M., Wurdack, K., Xiang, J.Q.Y., Zmarzty, S., Grp, A.P., 2003. An update of the Angiosperm phylogeny group classification for the orders and families of flowering plants: APG II. *Botanical Journal of the Linnean Society* 141, 399–436.
- Castro-Diez, P., Montserrat-Martí, G., 1998. Phenological pattern of fifteen Mediterranean phanerophytes from *Quercus ilex* communities of NE-Spain. *Plant Ecology* 139, 103–112.
- Cowling, R.M., Ojeda, F., Lamont, B.B., Rundel, P.W., Lechmere-Oertel, R., 2005. Rain-fall reliability, a neglected factor in explaining convergence and divergence of plant traits in fire-prone Mediterranean-climate ecosystems. *Global Ecology and Biogeography* 14, 509–519.
- Christensen, J.H., Hewitson, B., Busuioac, A., Chen, A., Gao, X., Held, I., Jones, R., Kolli, R.K., Kwon, W.T., Laprise, R., Magaña Rueda, V., Mearns, L., Menéndez, C.G., Räisänen, J., Rinke, A., Sarr, A., Whetton, P., 2007. Regional climate projections. Contribution of working group I to the fourth assessment report of the intergovernmental panel on climate change. In: Solomon, S., Qin, D., Manning, M., Chen, Z., Marquis, M., Averyt, K.B., Miller, M.T.H.L. (Eds.), *In Climate Change 2007: The Physical Science Basis*. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, pp. 847–940.
- Daehler, C.C., 2003. Performance comparisons of co-occurring native and alien invasive plants: implications for conservation and restoration. *Annual Review of Ecology Evolution and Systematics* 34, 183–211.
- Felsenstein, J., 1985. Phylogenies and the comparative method. *American Naturalist* 125, 1–15.
- Ghouil, H., Montpied, P., Epron, D., Ksontini, M., Hanchi, B., Dreyer, E., 2003. Thermal optima of photosynthetic functions and thermostability of photochemistry in cork oak seedlings. *Tree Physiology* 23, 1031–1039.
- Gimeno, T.E., Pias, B., Lemos-Filho, J.P., Valladares, F., 2009. Plasticity and stress tolerance override local adaptation in the responses of Mediterranean holm oak seedlings to drought and cold. *Tree Physiology* 29, 87–98.
- Godoy, O., Castro-Diez, P., Valladares, F., Costa-Tenorio, M., 2009. Different flowering phenology of alien invasive species in Spain: evidence for the use of an empty temporal niche? *Plant Biology* 11, 803–811.
- Gu, L., Hanson, P.J., Post, W.M., Kaiser, D.P., Yang, B., Nemani, R., Pallardy, S.G., Meyers, T., 2008. The 2007 Eastern US Spring Freeze: increased cold damage in a warming world? *BioScience* 58, 253–262.
- Hargreaves, G.H., Samani, Z.A., 1985. Reference crop evapotranspiration from temperature. *Applied Engineering in Agriculture* 1, 96–99.
- Hamilton, M.A., Murray, B.R., Cadotte, M.W., Hose, G.C., Baker, A.C., Harris, C.J., Licari, D., 2005. Life-history correlates of plant invasiveness at regional and continental scales. *Ecology Letters* 8, 1066–1074.
- Havaux, M., 1992. Stress tolerance of photosystem II in vivo. Antagonistic effects of water, heat and photoinhibition stresses. *Plant Physiology* 100, 424–432.
- Havaux, M., 1993. Rapid photosynthetic adaptation to heat stress triggered in potato leaves by moderately elevated temperatures. *Plant, Cell and Environment* 16, 461–467.
- Havaux, M., Tardy, F., 1996. Temperature-dependent adjustment of the thermal stability of photosystem II in vivo: possible involvement of xanthophyll-cycle pigments. *Planta* 198, 324–333.
- Hawkins, B.A., Field, R., Cornell, H.V., Currie, D.J., Guegan, J.-F., Kaufman, D.M., Kerr, J.T., Mittelbach, G.G., Oberdorff, T., O'Brien, E.M., Porter, E.E., Turner, J.R.G., 2003. Energy, water, and broad-scale geographic patterns of species richness. *Ecology* 84, 3105–3117.
- Herrera, C.M., 1992. Historical effects and sorting processes as explanations for contemporary ecological patterns, character syndromes in mediterranean woody plants. *American Naturalist* 140, 421–446.
- Hijmans, R.J., Cameron, S., Parra, J., Jones, P., Jarvis, A., Richardson, K., 2009. WorldClim. Global climate data. Versión 1.4 (release 3).
- Hüve, K., Bichele, I., Tobias, M., Niinemets, Ü., 2006. Heat sensitivity of photosynthetic electron transport varies during the day due to changes in sugars and osmotic potential. *Plant, Cell and Environment* 29, 212–228.
- van Kleunen, M., Weber, E., Fischer, M., 2010. A meta-analysis of trait differences between invasive and non-invasive plant species. *Ecology Letters* 13, 235–245.
- Krause, G.H., Winter, K., Krause, B., Jahns, P., García, M., Aranda, J., Virgo, A., 2010. High-temperature tolerance of a tropical tree, *Ficus insipida*: methodological reassessment and climate change considerations. *Functional Plant Biology* 37, 890–900.
- Knight, C.A., Ackerly, D.D., 2001. Correlated evolution of chloroplast heat shock protein expression in closely related plant species. *American Journal of Botany* 88, 411–418.
- Knight, C.A., Ackerly, D.D., 2003. Evolution and plasticity of photosynthetic thermal tolerance, specific leaf area and leaf size: congeneric species from desert and coastal environments. *New Phytologist* 160, 337–347.
- Krause, G.H., Weis, E., 1991. Chlorophyll fluorescence and photosynthesis: the basics. *Annual Review of Plant Physiology and Plant Molecular Biology* 42, 313–349.
- Ladjal, M., Epron, D., Ducrey, M., 2000. Effects of drought preconditioning on thermo-tolerance of photosystem II and susceptibility of photosynthesis to heat stress in cedar seedlings. *Tree Physiology* 20.
- Loveys, B.R., Schurwater, I., Pons, T.L., Fitter, A.H., Atkin, O.K., 2002. Growth temperature influences the underlying components of relative growth rate: an investigation using inherently fast- and slow-growing plant species. *Plant, Cell and Environment* 25, 975–987.
- Mitrakos, K.A., 1980. A theory for Mediterranean plant life. *Acta Oecologica* 1, 245–252.
- Nicotra, A.B., Chazdon, R.L., Schlichting, C.D., 1997. Patterns of genotypic variation and phenotypic plasticity of light response in two tropical Piper (Piperaceae) species. *American Journal of Botany* 84, 1542–1552.
- Niinemets, Ü., 2001. Global-scale climatic controls of leaf dry mass per area, density, and thickness in trees and shrubs. *Ecology* 82, 453–469.
- Niinemets, U., Valladares, F., 2006. Tolerance to shade, drought, and waterlogging of temperate Northern Hemisphere trees and shrubs. *Ecological Monographs* 76, 521–547.
- Niinemets, U., Portsmuth, A., Tena, D., Tobias, M., Matesanz, S., Valladares, F., 2007. Do we underestimate the importance of leaf size in plant economics? Disproportional scaling of support costs within the spectrum of leaf physiognomy. *Annals of Botany* 100, 283–303.
- Ogaya, R., Peñuelas, J., 2004. Phenological patterns of *Quercus ilex*, *Phillyrea latifolia*, and *Arbutus unedo* growing under field experimental drought. *Ecoscience* 11, 263–270.
- Oliveira, G., Peñuelas, J., 2004. Effects of winter cold stress on photosynthesis and photochemical efficiency of PSII of the Mediterranean *Cistus albidus* L. and *Quercus ilex* L. *Plant Ecology* 175, 179–191.
- Pastenes, C., Pimentel, P., Lillo, J., 2005. Leaf movements and photoinhibition in relation to water stress in field-grown beans. *Journal of Experimental Botany* 56, 425–433.
- Pyšek, P., Richardson, D.M., 2007. Traits Associated with invasiveness in alien plants: where do we stand? In: Caldwell, M.M. (Ed.), *Biological Invasions*. Springer, Berlin Heidelberg, pp. 97–125.
- Pyšek, P., Richardson, D.M., Rejmánek, M., Webster, G.L., Williamson, M., Kirschner, J., 2004. Alien plants in checklist and floras: towards better communication between taxonomist and ecologist. *Taxon* 53, 131–143.
- Qiu, N., Lu, C., 2003. Enhanced tolerance of photosynthesis against high temperature damage in salt-adapted halophyte *Atriplex centralasiatica* plants. *Plant, Cell and Environment* 26, 1137–1145.
- Reich, P.B., Oleksyn, J., 2004. Global patterns of plant N and P in relation to temperature and latitude. *Proceedings of the National Academy of Sciences of the United States of America* 101, 11001–11006.
- Richardson, D.M., Pyšek, P., Rejmánek, M., Barbour, M.G., Panetta, F.D., West, C.J., 2000. Naturalization and invasion of alien plants: concepts and definitions. *Diversity and Distributions* 6, 93–107.
- Roth-Nebelsick, A., 2001. Computer-based analysis of steady-state and transient heat transfer of small-sized leaves by free and mixed convection. *Plant, Cell and Environment* 24, 631–640.
- Salvucci, M.E., Crafts-Brandner, S.J., 2004. Relationship between the heat tolerance of photosynthesis and the thermal stability of rubisco activase in plants from contrasting thermal environments. *Plant Physiology* 134, 1460–1470.
- Samani, Z.A., 2000. Estimating solar radiation and evapotranspiration using minimum climatological data. *Journal of Irrigation and Drainage Engineering* 126, 265–267.
- Sánchez-Gómez, D., Valladares, F., Zavala, M.A., 2006. Performance of seedlings of Mediterranean woody species under experimental gradients of irradiance and water availability: trade-offs and evidence for niche differentiation. *New Phytologist* 170, 795–806.
- Sanz Elorza, M., Dana Sanchez, D., Sobrino Vesperinas, E., 2004. Atlas de las Plantas Alóctonas Invasoras en España. Ministerio de Medio Ambiente, Madrid.
- Schlichting, C.D., 1989. Phenotypic integration and environmental change. What are the consequences of differential phenotypic plasticity of traits. *Bioscience* 39, 460–464.
- Soltis, D.E., Soltis, P.S., Chase, M.W., Mort, M.E., Albach, D.C., Zanis, M., Savolainen, V., Hahn, W.H., Hoot, S.B., Fay, M.F., Axtell, M., Swensen, S.M., Prince, L.M., Kress, W.J., Nixon, K.C., Farris, J.S., 2000. Angiosperm phylogeny inferred from 18S rDNA, rbcL, and atpB sequences. *Botanical Journal of the Linnean Society* 133, 381–461.
- Stancato, G.C., Mazzafera, P., Buckeridge, M.S., 2001. Effect of a drought period on the mobilisation of non-structural carbohydrates, photosynthetic efficiency and water status in an epiphytic orchid. *Plant Physiology and Biochemistry* 39, 1009–1016.

- Thuiller, W., Richardson, D.M., Pyšek, P., Midgley, G.F., Hughes, G.O., Rouget, M., 2005. Niche-based modelling as a tool for predicting the risk of alien plant invasions at a global scale. *Global Change Biology* 11, 2234–2250.
- Valéry, L., Fritz, H., Lefeuvre, J.C., Simberloff, D., 2008. In search of a real definition of the biological invasion phenomenon itself. *Biological Invasions* 10, 1345–1351.
- Valladares, F., Percy, R., 1997. Interactions between water stress, sun-shade acclimation, heat tolerance and photoinhibition in the sclerophyll *Heteromeles arbutifolia*. *Plant, Cell and Environment* 20, 25–36.
- Valladares, F., Balaguer, L., Martinez-Ferri, E., Perez-Corona, E., Manrique, E., 2002. Plasticity, instability and canalization: is the phenotypic variation in seedlings of sclerophyll oaks consistent with the environmental unpredictability of Mediterranean ecosystems? *New Phytologist* 156, 457–467.
- Wikstrom, N., Savolainen, V., Chase, M.W., 2001. Evolution of the angiosperms: calibrating the family tree. *Proceedings of the Royal Society of London Series B: Biological Sciences* 268, 2211–2220.
- Wright, I.J., Reich, P.B., Westoby, M., Ackerly, D.D., Baruch, Z., Bongers, F., Cavender-Bares, J., Chapin, F.S., Cornelissen, J.H.C., Diemer, M., Flexas, J., Garnier, E., Groom, P.K., Gulias, J., Hikosaka, K., Lamont, B.B., Lee, T., Lee, W., Lusk, C., Midgley, J.J., Navas, M.L., Niinemets, U., Oleksyn, J., Osada, N., Poorter, H., Poot, P., Prior, L., Pyankov, V.I., Roumet, C., Thomas, S.C., Tjoelker, M.G., Veneklaas, E.J., Villar, R., 2004. The world-wide leaf economics spectrum. *Nature* 428, 821–827.
- Wright, I.J., Falster, D.S., Pickup, M., Westoby, M., 2006. Cross-species patterns in coordination between leaf and stem traits, and their implication for plant hydraulics. *Physiologia Plantarum* 127, 445–456.
- Xu, S.M., Liu, L.X., Woo, K.C., Wang, D.L., 2007. Changes in photosynthesis, xanthophyll cycle, and sugar accumulation in two North Australia tropical species differing in leaf angles. *Photosynthetica* 45, 348–354.