RESEARCH PAPER

Acclimation of photosystem II to high temperature in two *Wedelia* species from different geographical origins: implications for biological invasions upon global warming

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Abstract

More intense, more frequent, and longer heat waves are expected in the future due to global warming, which could have dramatic ecological impacts. However, few studies have involved invasive species. The aims of this study were to examine the effect of extreme heating (40/35 °C for 30 d) on the growth and photosynthesis of an alien invasive species *Wedelia trilobata* and its indigenous congener (*Wedelia chinensis*) in South China, and to determine the development of this invasive species and its potential adaptive mechanism. In comparison with *W. chinensis*, *W. trilobata* suffered less inhibition of the relative growth rate (RGR) and biomass production due to high temperature, which was consistent with the changes of photosystem II (PSII) activity and net photosynthetic rate (P_n). High temperature caused a partial inhibition of PSII, but the adverse effect was more severe in *W. chinensis*. Measurement of the minimum fluorescence (F_o) versus temperature curves showed that *W. trilobata* had a higher inflexion temperature of F_o (T_i), indicating greater thermostability of the photosynthetic apparatus. Moreover, comparisons of absorbed light energy partitioning revealed that *W. trilobata* increased xanthophyll-dependent thermal dissipation (Φ_{NPQ}) under high temperature, while retaining the higher fraction of absorbed light allocated to photochemistry (Φ_{PSII}) relative to *W. chinensis*. The results suggest that the invasive *W. trilobata* has a high thermostability of its photosynthetic apparatus and an effective regulating mechanism in energy partitioning of PSII complexes to minimize potential damage and to retain greater capability for carbon assimilation. These factors confer greater heat stress tolerance compared with the native species. Therefore, the invasive *W. trilobata* may become more aggressive with the increasingly extreme heat climates.

Key words: Chlorophyll fluorescence, high temperature, invasive species, photosynthesis, thermotolerance.

Introduction

Invasion by exotic species is a major component of global environmental change (Vitousek *et al.*, 1996; Dukes and Mooney, 1999; Vilà *et al.*, 2006; Thuiller *et al.*, 2007), and a likely cause of tremendous damage to worldwide economies and ecosystems (Pimentel *et al.*, 2005). In addition, exotic species are capable of interacting with other elements of global change to alter biodiversity and ecosystem pro-

cesses in invaded habitats (Dukes and Mooney, 1999; Ziska, 2003; Vilà *et al.*, 2006).

Global warming is one of the most important characteristics of global environmental change. In the last century, global mean temperatures have already risen by 0.74 °C, currently continuing to increase at ~0.1 °C per decade (IPCC, 2007). Some exotic species, such as *Spartina anglica*

and *Cylindrospermopsis raciborskii* (Woloszynska), and several tropical and warm-temperate agriculture weeds have been reported to exploit the new environmental conditions to expand their distribution into areas of high elevation (Patterson, 1995; Briand *et al.*, 2004; Nehring and Hesse, 2008). In addition to a mean increase in annual temperature, extreme heat events (e.g. a heat wave or hot days) will become more frequent and severe over most land areas under future climate scenarios (Wagner, 1996; IPCC, 2007). These extreme events, though ephemeral, can cause dramatic ecological impacts which might be significantly greater than those associated with mean temperature increases (Karl *et al.*, 1997). Sage and Kubien (2003) have reported that in natural systems, the significance of climate warming for C_4 vegetation depends less on the mean increase in global temperature and more on the spatial and temporal variation of the temperature increase. Some researchers have examined the responses of plant species to heat waves (White *et al.*, 2000, 2001; García-Plazaola *et al.*, 2008; Wang *et al.*, 2008). However, few studies have involved invasive species, so the ecological impact of extreme heat events on plant invasions remains unclear.

Wedelia trilobata (L.) Hitchc. is a creeping herb native to tropical Central America, which has invaded many areas of the tropics and subtropics (Thaman, 1999). It has been listed as one of the world's 100 worst invasive alien species (IUCN, 2001). In the 1970s, *W. trilobata* was introduced into China as an ornamental groundcover, but it rapidly escaped from gardens to roadsides and plantations (Li and Xie, 2002). Thus, it has become recognized as a serious weed in southern China (Wu *et al.*, 2005*b*). Previous studies suggested that fast dispersal by vegetative propagation (Wu *et al.*, 2005*a*), high photosynthetic activity (Wu and Hu, 2004; Liu and Li, 2005), allelopathic potential (Vieira *et al.*, 2001; Nie *et al.*, 2004; Zhang *et al.*, 2004), and energy-use efficiency (Song *et al.*, 2009) are the principal factors explaining its successful invasion.

In recent years, the frequency, duration, and severity of extreme heat stress in southern China have increased, accompanied by rising mean temperature. In this scenario, *W. trilobata* encounters severe high temperature stress in summer, as does its indigenous congener. The capacity for ecophysiological acclimation to heat stress might be critical for the survival and dispersal of plant seedlings. Since *W. trilobata* originates from tropical regions, it was hypothesized that it might have developed a suite of physiological characters that confer on it a greater ability to withstand acute heat events.

High temperatures adversely affect plant growth in a number of ways, with photosynthesis considered as one of the most heat-sensitive processes (Berry and Björkman, 1980; Schrader *et al.*, 2004). Photosystem II (PSII) is the critical site of damage by high temperatures (Allakhverdiev *et al.*, 2008). High temperature >45 °C is known to damage PSII (Yamane *et al.*, 1998; Sharkey, 2005). Moderate heat stress (e.g. 35–42 °C) has been reported to increase PSI cyclic electron flow (Pastenes and Horton, 1996; Bukhov *et al.*, 1999; Zhang and Sharkey, 2009) and thylakoid proton conductance (Schrader *et al.*, 2004) and reduces the photosynthetic rate (Feller *et al.*, 1998), but its effect on PSII is only partial inhibition (Sun *et al.*, 2007) and it is reversible (Sharkey, 2005). Possibly plants possess an effective regulatory system to protect photosynthesis from moderately high temperatures that do not damage PSII, such as down-regulation of PSII and thermal dissipation (Kato *et al.*, 2003; Kornyeyev *et al.*, 2004). Quantifying the fate of absorbed light energy is of importance for studying the response of the photosynthetic apparatus to environmental factors and the regulatory mechanisms involved in acclimation (Kornyeyev and Hendrikson, 2007). However, few studies have evaluated the effect of moderately high temperature on these aspects.

Comparison of invasive species and native species is an efficient approach to identifying mechanisms of invasive plant success, and thus provides insight useful for predicting the development of local communities (Goldberg, 1987; Mack, 1996). In this study, the effects of simulated heat stress on the invasive *W. trilobata* and its indigenous congener [*Wedelia chinensis* (Osbeck.) Merr.] were compared. The main objectives were (i) to determine how simulated heat stress affects the growth of invasive *W. trilobata* and its indigenous congener *W. chinensis*, with a view to predicting the development of this invasive species in the future; and (ii) to characterize the acclimation of PSII in these two species to high temperature, in order to determine whether there is an existing specific photosynthetic regulatory mechanism in this invasive species enabling it to withstand high temperature.

Materials and methods

Plant materials and high temperature treatments

Both plant species, *W. trilobata* and *W. chinensis*, were collected from Guangzhou, Guangdong Province, China. They share similar morphologies and life histories, and overlap in range. In contrast to *W. trilobata*, *W. chinensis* grows slowly and has not been found harmful to native plants or habitats in China. The experimental plant materials were grown from stem cuttings ~5 cm long. After 2–4 weeks of propagation, uniform seedlings (~10 cm tall) were selected and transplanted into pots containing a 1:1:1 mixture of river sand, pool mud, and peat. The high temperature treatments simulating either an extreme heat event or mild temperatures were conducted by using two growth chambers (RXZ-500, Ningbo Instruments, China). According to the reports on climate change of Guangdong, the number of high temperature days (≥35 °C) is >20 per year, and the maximum temperature can approach 40 °C in the summer (Composing Team for Assessment Report on Climate Change of Guangdong, 2007). The high temperature treatment was maintained at 40/35 °C (day/night), and the control was set at 28/23 °C (day/night), representing the annual average temperature of Guangzhou. The high temperature treatment lasted for 30 d. For each species, 12 individuals were randomly assigned to one growth chamber. In both chambers, the photosynthetically active irradiance was ~200 μmol m^{-2} s^{-1} with a photoperiod of 12 h, and the relative humidity was ~65%. All pots were watered daily to maintain the soil water content at ~50%.

Growth measurements

Prior to the exposure to high temperature, six additional seedlings of each species of the same size as the experimental seedlings were

harvested to determine the initial biomass. After 30 d of treatment, all plant materials were harvested and total biomass per individual was determined after drying for 72 h in an oven set at 70 °C. The relative growth rate (RGR) was calculated as the net dry matter increase per unit dry mass per day (mg g^{-1} d^{-1}) averaged over 30 d.

Chlorophyll fluorescence and gas exchange measurements

During the high temperature treatment, chlorophyll (Chl) fluorescence parameters were measured *in situ* continually at 2–3 d intervals with a portable pulse-modulated fluorimeter PAM-2100 (Walz, Efeltrich, Gemany). Leaf samples were dark adapted for 15 min before measurements of the minimum (F_o) and maximal fluorescence (F_m). The maximal photochemical efficiency of PSII (F_v/F_m) was calculated as $F_v/F_m=(F_m-F_o)/F_m$ (Schreiber *et al.*, 1986). The steady-state (F_s) and maximum fluorescence (F_m') in the light-adapted state were measured under an actinic light irradiance of 200 µmol m^{-2} s^{-1}. The effective photochemical efficiency of PSII (Φ_{PSII}) was calculated as $\Phi_{PSII}=(F_m'-F_s)/F_m'$ (Genty *et al.*, 1989). The total electron transport rate through PSII (ETR) was estimated according to Krall and Edwards (1992): $ETR=\Phi_{PSII}\times PPFD\times A\times 0.5$, where A is the leaf absorptance, which was estimated as 0.84. The factor 0.5 was based on the assumption of an equal distribution of photons between PSI and PSII. Incident irradiance was measured with a quantum sensor. After heat stress, gas exchange was measured concurrently with Chl fluorescence on the same leaves with a portable photosynthesis measuring system (Li-6400, Portable Photosynthesis System, Li-Cor, Lincoln, NE, USA).

Partitioning of the absorbed light energy was estimated according to the model proposed by Hendrickson *et al.* (2004). The allocation of photons absorbed by the PSII antennae to photosynthetic electron transport and PSII photochemistry was estimated as $\Phi_{PSII}=1-(F_s/F_m')$. The quantum efficiency of regulated ΔpH- and/or xanthophyll-dependent non-photochemical dissipation processes within the PSII antennae (Φ_{NPQ}) was calculated as: $\Phi_{NPQ}=(F_s/F_m')-(F_s/F_m)$. Constitutive non-photochemical energy dissipation and fluorescence was calculated as: $\Phi_{f,D}=F_s/F_m$ (Cailly *et al.*, 1996).

The temperature-dependent increase in minimal florescence in the dark (F_o versus T curve) was determined as described by Lin *et al.* (2005). The third and fourth mature leaves from 4-week-old plants grown at the ambient temperature were used for this experiment. Before the measurement of F_o, leaf samples were dark adapted for 15 min at 25 °C. Then leaf discs were placed in a water bath of 25–80 °C, in which the temperature was increased continuously at 2 °C min^{-1}, and the corresponding F_o was recorded at 0.02 s intervals with a PAM-2100. The inflexion temperature at which F_o began to rise sharply (T_i) and the peak temperature (T_p) were recorded.

Measurements of physiological characteristics

After the high temperature treatment, the mature and fully expanded leaves were collected for pigments analysis. The Chl content was quantified using the procedure described by Arnon (1949). Xanthophyll cycle pigments were extracted with ice-cold 100% acetone from leaf tissue that had been frozen in liquid nitrogen immediately after detachment and ground in a mortar. The extract was analysed using an HPLC system (Agilent 1100LC, Germany) according to the method described by Gilmore and Yamamoto (1991). The de-epoxidation state (DES) of the xanthophyll cycle was calculated as (Z+0.5×A)/(V+A+Z), where V, A, and Z correspond to the concentration of violaxanthin, antheraxanthin, and zeaxanthin, respectively (Gilmore and Yamamoto, 1993).

In order to measure the cell membrane leakage rate, leaves [0.2 g fresh weight (FW)] of the two species were immersed in double-distilled water for 1.5 h at room temperature, followed by a 30 min boiling treatment. The conductivity of a solution of leaked electrolytes before (L_t) and after boiling (L_o) was determined using a DDS-11C conductometer (Shanghai Dapu Instruments, Shanghai, China). Membrane leakage rate was defined as L_t/L_o and expressed as a percentage (Vieira Santos *et al.*, 2001).

For extraction and determination of heat-stable protein, leaves (0.5 g FW) were ground and extracted in an ice bath with 5 ml of pre-cooled phosphate buffer (pH 7.0). The homogenate was heated in boiling water for 15 min, and then placed into an ice bath to cool for 30 min. After centrifugation at 4 °C, 13 000 g for 15 min, the content of the heat-stable protein in the supernatant was measured using Coomassie brilliant blue (Bradford, 1976). The content of the heat-stable protein was expressed as a percentage of the total soluble protein.

In order to assess total antioxidative capability, 50% ethanol extracts were prepared from the leaves of the two *Wedelia* species (0.1 g FW), followed by centrifugation at 4 °C and 5000 g for 15 min. The supernatant was mixed with coloured DPPH· (1.1-diphenyl-2-picrylhy-drazyl) solution. The decrease of coloured DPPH· solution absorbance was measured at 525 nm using a spectrophotometer (UV-2450, Shimadzu, Japan) as described by Peng *et al.* (2000). Total antioxidative capability was expressed as the scavenging amount of an organic free radical DPPH· per g FW of leaves.

Statistical analysis

All statistical tests were performed using SPSS 11.5 software (SPSS Inc., USA). A two-way analysis of variance (ANOVA) was performed to evaluate the main effects and interactive effect of high temperature and species on measured variables. Treatment means were compared to determine if means of the dependent variable were significant at the 0.05 probability level with the least significant difference (LSD) *post-hoc* analysis.

Results

RGR was significantly affected by species, high temperature, and their interaction (Table 1). *Wedelia chinensis* exposed to high temperature exhibited a significant decrease in RGR. However, the RGR of *W. trilobata* was not significantly changed by high temperature (Fig. 1A). High temperature-induced changes in RGR were associated with a consistent trend in the total biomass. The total biomass was affected significantly by species and high temperature (Table 1). A significant decrease of total

Table 1. Results from two-way ANOVA (*F* value) for treatment effects on plant individual variables

Variables	Species	High temperature	Species×high temperature
Biomass	104.1**	8.08**	2.23ns
RGR	56.34**	34.98**	20.77**
F_v/F_m	33.21**	300.0**	16.96**
F_o	16.72**	195.8**	14.64**
ETR	0.77ns	773.5**	4.32*
Φ_{PSII}	1.99ns	350.2**	2.88ns
Φ_{NPQ}	0.55ns	220.7**	0.22ns
$\Phi_{f,D}$	19.64**	1.22ns	18.54**
P_n	6.03*	37.30**	14.22**

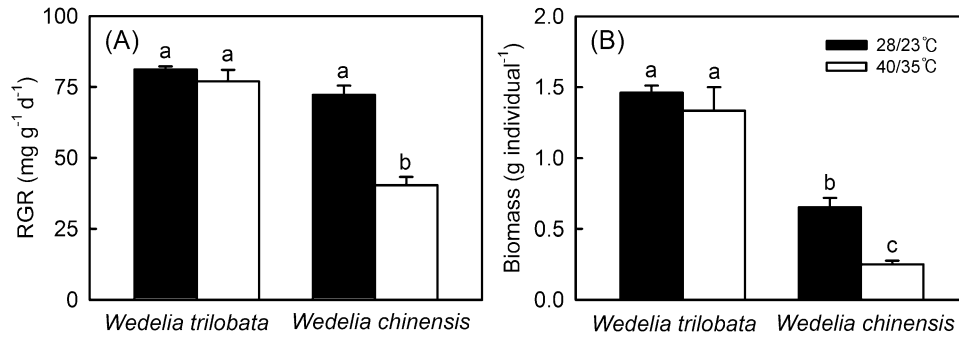Significance levels: ns, $P >0.05$; *$P <0.05$; **$P <0.01$.

Fig. 1. Effects of simulated heat events on the relative growth rate (RGR) (A) and total biomass (B) of the invasive *Wedelia trilobata* and the native *Wedelia chinensis*. Error bars represent 1 SE. Means with a common letter do not differ from each other based on LSD *post-hoc* analysis at the P=0.05 level.

Table 2. Total chlorophyll (Chl) content, Chl *a/b* ratio, carotenoid content, heat-stable protein content, membrane leakage rate, and DPPH· scavenging capacity in leaves of invasive *Wedelia trilobata* and native *Wedelia chinensis* at 28/23 °C and 40/35 °C.

Variable	*Wedelia trilobata*		*Wedelia chinensis*	
	28/23 °C	**40/35 °C**	**28/23 °C**	**40/35 °C**
Total Chl (µg cm^{-2})	35.29±1.48 c	52.80±1.43 ab	54.72±2.34 a	48.19±1.23 b
Chl *a/b* ratio	2.77±0.02 a	2.55±0.01 b	2.70±0.08 a	2.37±0.01 c
Carotenoid (µg cm^{-2})	3.30±0.13 b	4.54±0.09 a	4.74±0.21 a	4.78±0.14 a
Heat-stable protein content (%)	10.37±0.60 a	9.35±0.70 ab	9.32±0.20 ab	7.83±0.10 b
Membrane leakage rate (%)	14.10±0.65 c	17.17±0.25 b	12.80±0.23 d	19.78±0.24 a
DPPH· scavenging capacity (mg DPPH· g^{-1} FW)	44.16±2.19 a	31.09±1.90 b	25.46±1.11 c	31.07±1.27 b

Data are means ±1 SE. For each variable, means labelled with the same letter are not significantly different according to LSD *post-hoc* analysis at the P=0.05 level.

biomass was found in *W. chinensis* by the high temperature treatment, whereas no significant change occurred in *W. trilobata* (Fig. 1B).

As shown in Table 2, high temperature influenced various physiological processes in these two species. High temperature decreased the total Chl content of *W. chinensis*, but increased that of *W. trilobata*. The Chl *a/b* ratio was decreased under 40/35 °C in both species. It indicated that the Chl, especially Chl *a*, was sensitive to high temperature. In addition, high temperature increased the carotenoid content of *W. trilobata*, but did not affect that of *W. chinensis*. Exposure to high temperature resulted in a trend toward a decrease in the content of heat-stable proteins, but this was not significantly different in the two species at high temperature. The membrane leakage rate is a relevant index reflecting the degree of impaired membrane function. High temperature induced an increase in the membrane leakage rate of both *Wedelia* species, suggesting that their plasma membranes, and possibly also their chloroplast envelope membranes, were damaged to some extent. After high temperature treatment, the membrane leakage rate of *W. chinensis* was significantly higher than that of *W. trilobata*. Measurement of scavenging of the DPPH· free radicals is a rapid, simple, sensitive, and practical assay for evaluating antioxidant capacity in plants (Peng *et al.*, 2000). In this respect, these two species exhibited different responses to high temperature. The DPPH· scavenging capacity of invasive *W. trilobata* declined

by 29.6%, whereas that of *W. chinensis* exhibited an increase of 22.0%. After the heat treatment, these two species reached a similar level of DPPH· scavenging capacity.

The maximal photochemical efficiency of PSII (F_v/F_m) and the minimum fluorescence (F_o) were significantly affected by species, high temperature, and their interactive effect (Table 1). Upon exposure to high temperature, an obvious decreasing trend of F_v/F_m was observed in both *Wedelia* species (Fig. 2A). Within the first 2 d, F_v/F_m underwent a sudden decline, followed by a slight recovery between 3 d and 12 d, and a continuous decrease beyond 12 d. Although there was no significant difference between the two species before the heat treatment, high temperature magnified the discrepancy of F_v/F_m between them. After treatment for 30 d, *W. trilobata* exhibited higher F_v/F_m than *W. chinensis* (P <0.001). While F_v/F_m decreased, these two species showed an increase in F_o with the duration of heat treatment (Fig. 2B). After the heat treatment for 30 d, F_o was increased by 31.3% and 48.2% in *W. trilobata* and *W. chinensis*, respectively.

High temperature had significant effects on Φ_{PSII} and ETR (Table 1). Both species exhibited a similar decreasing trend in Φ_{PSII} and ETR (Fig. 2C, D). The dynamic changes of Φ_{PSII} and ETR during heat treatment seemed to involve two stages of alteration. In the first stage (within the first 12 d), Φ_{PSII} and ETR declined slowly and there was no significant difference between these two species. In the second stage (beyond 12 d), *W. chinensis* exhibited
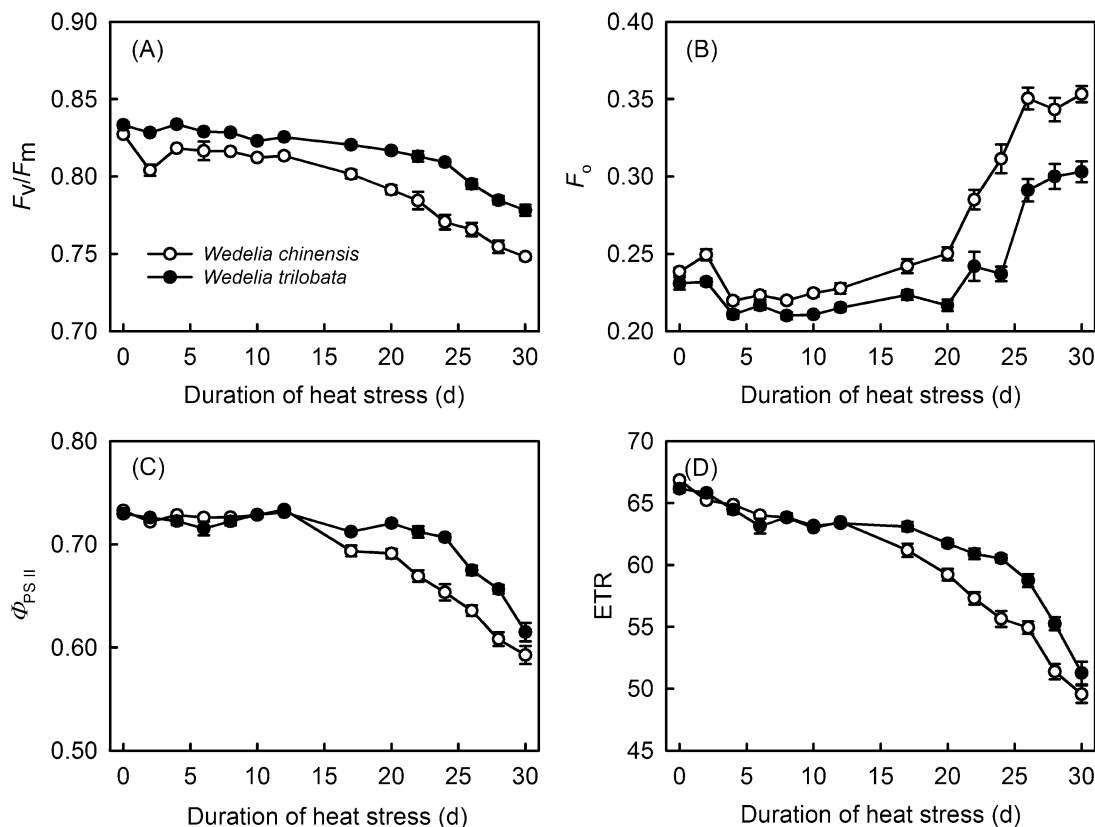
Fig. 2. Changes in the maximal photochemical efficiency of PSII (F_v/F_m) (A), the minimum fluorescence (F_o) (B), the effective photochemical efficiency of PSII (Φ_{PSII}) (C), and the total electron transport rate (ETR) (D) of invasive *Wedelia trilobata* and the native *Wedelia chinensis* during heat stress. Error bars represent 1 SE.

a relatively fast decrease in Φ_{PSII} and ETR compared with *W. trilobata*.

High temperature had significant effects on the energy partitioning of absorbed light to photochemical conversion (Φ_{PSII}) and thermal dissipation via ΔpH- and xanthophyll-dependent energy quenching (Φ_{NPQ}) (Table 1). The estimated energy partitioning of absorbed light to the various pathways indicated that the photochemistry fraction (Φ_{PSII}) was significantly decreased by high temperature ($P < 0.001$), while the thermal dissipation fraction (Φ_{NPQ}) was increased in both species ($P < 0.001$) (Fig. 3). In contrast, $\Phi_{f,D}$, the combined fraction of absorbed light energy dissipated constitutively as heat in a light-independent manner and as Chl fluorescence, showed different response patterns to high temperature in these two species. The invasive *W. trilobata* exhibited a significant decrease in $\Phi_{f,D}$ ($P = 0.045$), whereas the native *W. chinensis* showed an increase ($P = 0.001$) (Fig. 3). Under high temperature, the constitutive energy dissipation ($\Phi_{f,D}$) was significantly lower in *W. trilobata* than in *W. chinensis* ($P < 0.001$).

Leaf net photosynthetic rate (P_n) was affected significantly by species, high temperature, and their interaction (Table 1). Measured under high temperature, P_n showed a significant decrease of 82.0% in the native *W. chinensis*, while that in the invasive *W. trilobata* exhibited only a decrease of 21.9% which was not statistically significant (Fig. 4).

Exposure to high temperature resulted in a trend toward an increase of the total xanthophyll cycle pigments pool (V+A+Z) in both species (Fig. 5A). Furthermore, *W. trilobata* exhibited a significant increase in the DES of the xanthophyll cycle under high temperature, whereas no obvious change was observed in *W. chinensis* (Fig. 5B).

Figure 6A shows the F_o versus T curves in the leaves of *W. trilobata* and *W. chinensis*. With the continuous increase in temperature, F_o versus T curves showed a pattern similar to a parabola. The inflexion temperature (T_i) at which F_o began to rise sharply corresponds to the beginning of irreversible injury of photosynthetic membranes. *Wedelai trilobata* exhibited a higher inflexion temperature for F_o (48.0 °C) than *W. chinensis* (44.6 °C) (Fig. 6B). As shown in Fig. 6C, the peak temperature for F_o (T_p) in *W. trilobata* was also significantly higher (54.5 °C) than in *W. chinensis* (51.2 °C).

Discussion

Plant growth, photosynthesis, and photochemical efficiency

High temperature adversely affects various physiological processes which can reduce plant growth, survival, and, consequently, crop yield (Boyer, 1982). However, the relative inhibition varies greatly among plant species (Stasik
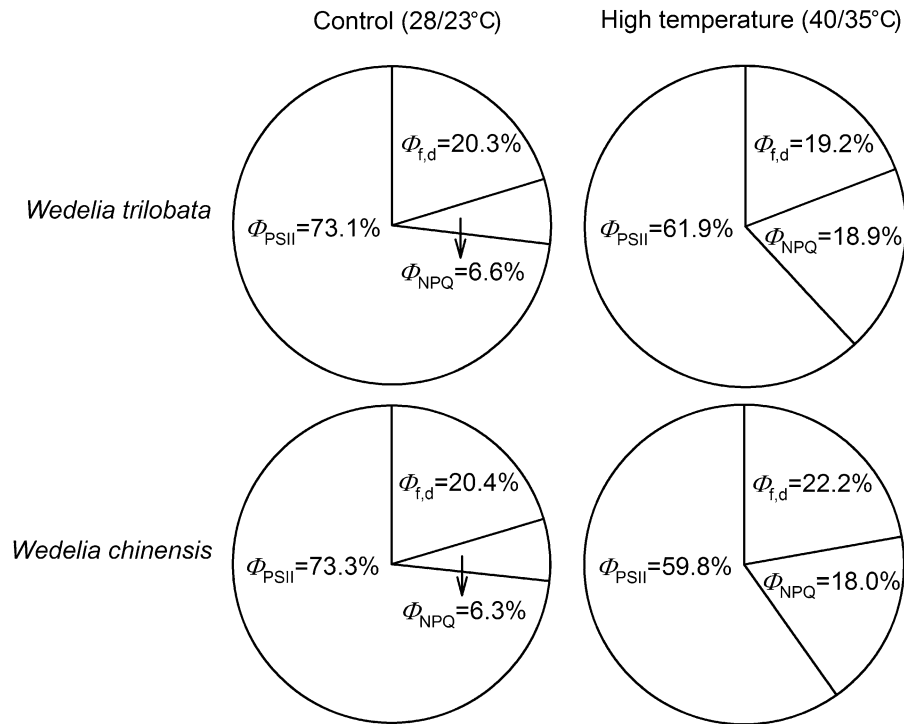
Control (28/23°C) High temperature (40/35°C)

Fig. 3. Effects of simulated heat events on the energy partitioning of absorbed light to photochemical process (Φ_{PSII}), ΔpH- and xanthophyll-dependent thermal dissipation (Φ_{NPQ}), and the sum of fluorescence and constitutive thermal dissipation ($\Phi_{f,D}$) of the invasive *Wedelia trilobata* and the native *Wedelia chinensis*.
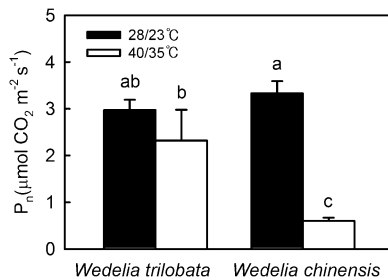

Fig. 4. Effects of simulated heat events on the net photosynthetic rate (P_n) of the invasive *Wedelia trilobata* and the native *Wedelia chinensis*. Error bars represent 1 SE. Means with a common letter do not differ from each other based on LSD *post-hoc* analysis at the $P=0.05$ level.

and Jones, 2007), such differences having important consequences for plant competition. Following a short-term exposure to high temperature, the growth of two *Wedelia* species was restricted to a varying degree. In particular, *W. chinensis* was more strongly inhibited in RGR and total biomass production compared with *W. trilobata* (Fig. 1). Photosynthesis is recognized as the most important determinant of plant growth; its responses and adaptation to the environment enable plants to exist and develop under various temperature conditions. Although high temperature decreased the net photosynthetic rate, *W. trilobata* exhibited a significantly higher P_n than *W. chinensis* (Fig. 4), which is consistent with the changes in RGR and biomass under heat stress.

High temperature did not decrease pigment content, and induced a smaller increase in membrane leakage in *W. trilobata* compared with *W. chinensis* (Table 2). These results indicated that the invasive *W. trilobata* had greater thermotolerance. Under field conditions, high temperature often accompanies high irradiance, drought, and other environmental stress, and thus heat stress is further aggravated. It is expected to intensify the negative impact on the native *W. chinensis* to some extent. Therefore, it was inferred that the invasive *W. trilobata* may become more aggressive in a warmer world in future.

PSII is particularly sensitive to high temperature (Berry and Björkman, 1980; Thompson *et al.*, 1989; Mamedov *et al.*, 1993). Exposure to a short-term high temperature treatment resulted in significant changes in PSII function in these two *Wedelia* species. An obvious decreasing trend of F_v/F_m was observed in these two species, while the value of F_o was increased under high temperature in the later stages of the treatment (Fig. 2). These changes in F_v/F_m and F_o are considered to be reliable diagnostic indicators of photoinhibition (Maxwell and Johnson, 2000). The present results are consistent with the evidence of Sun *et al.* (2007), which demonstrated that moderate high temperature stress causes a partial inhibition of PSII. Further, the adverse effect was more severe in native *W. chinensis*. After 30 d of heat stress, the invasive *W. trilobata* showed higher Φ_{PSII} and ETR than *W. chinensis* (Fig. 2C, D). All the above results show that the invasive species maintained relatively high activity of PSII under high temperature conditions.
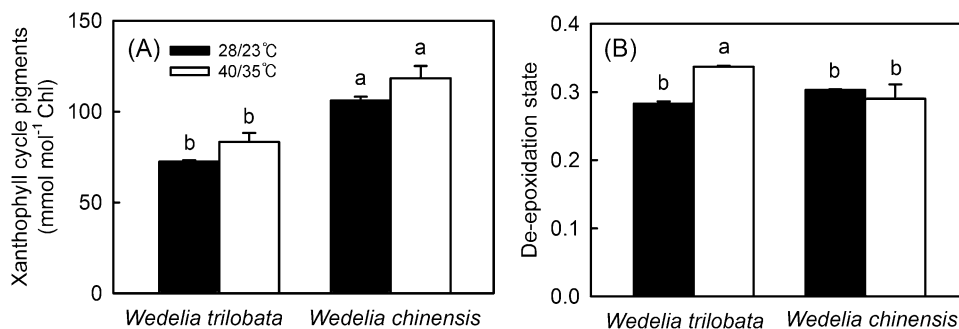
Fig. 5. Effects of simulated heat events on the total amounts of xanthophyll cycle pigments (A) and the de-epoxidation state (B) in the leaves of the invasive *Wedelia trilobata* and the native *Wedelia chinensis*. Error bars represent 1 SE. Means with a common letter do not differ from each other based on LSD *post-hoc* analysis at the $P=0.05$ level.
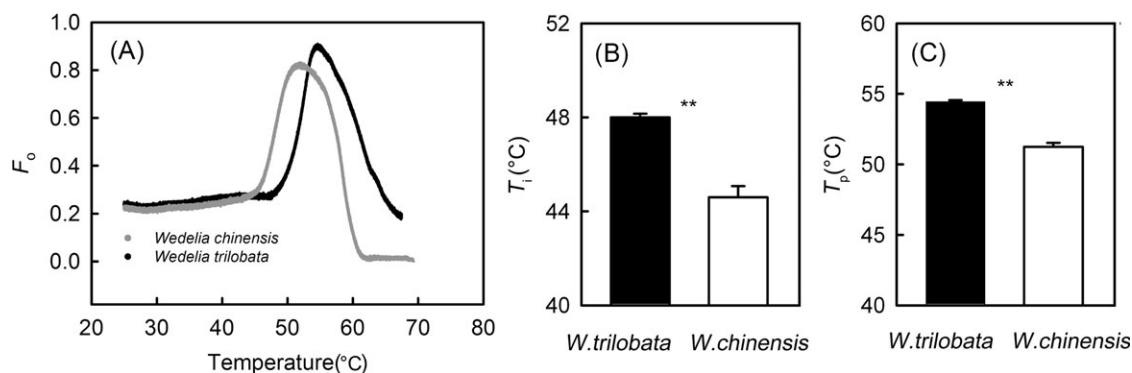

Fig. 6. The F_o versus. temperature curves (A), inflexion temperature (T_i) (B), and peak temperature (T_p) (C) in leaves of the invasive *Wedelia trilobata* and the native *Wedelia chinensis*. **$P <0.01$.

High thermal stability of the photosynthetic apparatus

High thermostability of the photosynthetic apparatus is conducive to the maintenance of PSII function at a high temperature. The temperature-dependent increase in minimal florescence in the dark (the F_o versus T curve) has been routinely used to estimate the thermostability of the photosynthetic apparatus (Schreiber and Berry, 1977; Schreiber and Bilger, 1987; Froux *et al.*, 2004; Weng and Lai, 2005). The temperature at which F_o began to rise sharply (T_i) is the critical point which indicates that irreversible injury has begun in the photosynthetic membrane (Georgieva and Yordanov, 1993). In this study, *W. trilobata* exhibited higher T_i than *W. chinensis* (Fig. 6B), suggesting that *W. trilobata* has a greater thermostability of its photosynthetic apparatus. Previous studies reported that moderate heat causes thylakoid membranes to become leaky (Schrader *et al.*, 2004). In the present study, *W. trilobata* displayed a smaller F_o increase following high temperature exposure than *W. chinensis* (Fig. 2B), suggesting a greater thermostability of thylakoid membranes in this invasive species. Additional experimental evidence is necessary to test this hypothesis further.

Partitioning of absorbed light energy

High temperature could accelerate photoinhibition by inducing an imbalance between light energy absorption and utilization. When the energy absorbed is in excess of the amount that can be used for carbon fixation, it could lead to the production of reactive oxygen species, which can damage the photosynthetic apparatus (Osmond, 1994; Müller *et al.*, 2001). In this study, the native *W. chinensis* exhibited significant decreases in total Chl content and the Chl *a*/*b* ratio under high temperature (Table 2), which is considered as an avoidance mechanism to reduce light absorption (Ishida *et al.*, 2000). In contrast, invasive *W. trilobata* had greater thermal tolerance; its leaves under high temperature became greener, with increased total Chl content (Table 2).

Thermal dissipation of excess excitation energy is an important photoprotective mechanism that plants have evolved to cope with excess absorbed light. Several models have been proposed to quantify the partitioning of total absorbed energy by PSII between the various processes of photochemistry and thermal dissipation (Demmig-Adams *et al.*, 1996; Hendrickson *et al.*, 2004; Kramer *et al.*, 2004), which is of significant importance for studying the response of the photosynthetic apparatus to high temperature and the regulatory mechanisms involved in acclimation. In the present study, the partitioning of the absorbed light to various pathways in control and heat-stressed plants was estimated according to the method of Hendrickson *et al.* (2004). For both *Wedelia* species, the fraction partitioned to photochemistry (Φ_{PSII}) decreased with rising temperature

(Fig. 3), presumably because Rubisco activase-mediated activation of Rubisco was inhibited at the higher temperature (Feller *et al.*, 1998). However, it is noteworthy that *W. trilobata* exhibited a relatively higher Φ_{PSII} following high temperature treatment (Fig. 2C), which resulted in more electron flow through PSII (ETR) compared with the native species (Fig. 2D). This result suggests that this invasive species had a greater capacity for energy utilization under high temperature. Any small improvement in energy utilization has a cumulative effect over a growing season, ameliorating the growth inhibition of invasive *W. trilobata* under high temperature.

Concomitantly, the fraction of the regulated ΔpH- and xanthophyll-dependent thermal dissipation process (Φ_{NPQ}) was significantly increased by high temperature in both *Wedelia* species (Fig. 3). This result is partly associated with the increased trend towards the xanthophyll cycle pigment pool (V+A+Z) (Fig. 5A). In comparison with *W. chinensis*, *W. trilobata* had a smaller xanthophyll pool size at both the control and elevated temperature. A previous study reported that plants with higher thermotolerance have a smaller xanthophyll pool size (Tsai and Hsu, 2009). Furthermore, high temperature enhanced the DES of the xanthophyll cycle in *W. trilobata*, whereas no obvious change was observed in *W. chinensis* (Fig. 5B). This differential effect resulted in the slightly higher Φ_{NPQ} fraction in *W. trilobata* (despite a smaller xanthophyll pool size) than that in *W. chinensis* under high temperature, which could offer more photoprotection to this invasive species. For *W. chinensis*, high temperature did not enhance its DES, but significantly increased its DPPH· scavenging capacity. It is deduced that the major protective mechanism against photodamage may be different for the two plant species; an elevated antioxidative capacity may be the predominant protective mechanism in *W. chinensis*, whereas *W. trilobata* might not need to enhance its antioxidative capacity. In this respect, it is interesting to note that in *W. trilobata* Φ_{PSII} decreased from 73.1% to 61.9% on transition to the high temperature regime (Fig. 3, top), a percentage decrease similar to that of P_n (Fig. 4, left). In contrast, in *W. chinensis* Φ_{PSII} decreased from 73.3% to 59.8% on acclimation to the high temperature regime (Fig. 3, bottom), a percentage decrease vastly smaller than that of P_n (Fig. 4, right). Conceivably, the 'missing' electron flow (the component of the ETR not coupled to P_n) that had gone through PSII in *W. chinensis* but not reflected in net carbon assimilation could have been diverted to antioxidative activity or other alternative electron pathways.

The proportion of constitutive thermal dissipation ($\Phi_{f,D}$) showed different responses to high temperature between these two species; $\Phi_{f,D}$ decreased slightly in *W. trilobata*, while it increased slightly in *W. cheninsis* (Fig. 3). Since $\Phi_{f,D}$ represents an inevitable energy loss, the invasive species seems to have an advantage over the native species in having a slightly lower $\Phi_{f,D}$ in the high temperature regime. The present results revealed that the invasive *W. trilobata* could regulate energy partitioning in PSII complexes to minimize damaging potential and retain a great capability for carbon assimilation. For the invasive *W. trilobata*, the effective regulation of energy partitioning around PSII might be an alternative acclimation mechanism to withstand high temperature.

In conclusion, the invasive *W. trilobata* has a relatively high thermostability of its photosynthetic apparatus and an adaptive regulation in energy partitioning in PSII complexes, which confer on it a greater ability than the native species to withstand heat stress. The invasive *W. trilobata* is likely to become more aggressive with the increasingly extreme heat climates. Furthermore, it should be noted that *W. trilobata* originates from the tropics of Central America, where the mean temperature is higher than in the invaded land in South China. In a long-term evolution process, *W. trilobata* has developed an adaptive capacity to withstand high temperature. Some previous studies have demonstrated that low elevation species have a higher thermal tolerance than high elevation species (Loik and Harte, 1996). The present results lead to the conclusion that those exotic species whose native habitats are warmer than their introduced ranges would have an advantage in an increasingly extreme heat climate.

Acknowledgements

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