Phytoprotection

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Résumé de l'article

Dans le sud de la Chine, l'espèce exotique envahissante *Ambrosia artemisiifolia* L. a été comparée à une espèce indigène compagne, *Urena lobata* L., afin d'évaluer le rôle protecteur potentiel fourni par les systèmes antioxydants de la feuille lors de l'acclimatation des plantes envahissantes soumises à différents taux de flux énergétique. Les activités antioxydants de la superoxyde dismutase (SOD), de la peroxydase (POD) et de la catalase (CAT) ont été examinées sous quatre régimes de flux énergétique : 10 % (ombrage dense), 30 % (faible flux énergétique), 55 % (flux énergétique moyen) et 100 % (flux énergétique complet). Le contenu en proline libre (Pro) et le taux de peroxydation lipidique en termes de malonaldéhyde (MDA), de réductase de glutathion (RG) et de polyphénols de thé (PT) ont également été évalués pour différents taux de flux énergétique. Les activités enzymatiques antioxydantes de SOD et de CAT et les contenus en MDA, RG et PT des deux espèces ont augmenté avec l'augmentation des taux de flux énergétique. L'espèce invasive *A. artemisiifolia* était en mesure de récupérer les radicaux d'oxygène plus efficacement à un taux de flux énergétique plus élevé grâce à une activité accrue de la CAT et des contenus de RG et PT plus élevés, bien que l'activité de la SOD dans la feuille n'ait pas beaucoup augmentée. Cette espèce exotique a également maintenu des fonctions physiologiques normales lorsque soumise à de faible taux de flux énergétique, ce qui pourrait être attribuable à l'augmentation de l'activité de la POD en lien avec une diminution du taux de flux énergétique. Une plus grande efficacité des réactions adaptatives des enzymes antioxidants pourrait protéger les plantes du stress relié au flux énergétique et contribuer à l'envahissement de l'*A. artemisiifolia* dans les régions tropicales et subtropicales.

Antioxidant response of the invasive herb *Ambrosia artemisiifolia* L. to different irradiance levels

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The exotic invasive *Ambrosia artemisiifolia* L. and a native co-occurring species in southern China, *Urena lobata* L., were compared to investigate the possible protective role of leaf antioxidant systems in the acclimation of invasive plants to different irradiance levels. Antioxidant activities of superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) were examined under four irradiance regimes: 10% (dense shade), 30% (low irradiance), 55% (medium irradiance) and 100% (full irradiance). Free proline (Pro) content and the rate of lipid peroxidation in terms of malondialdehyde (MDA) content, glutathione reductase (GR), and tea polyphenols (TP) were also assessed under the different irradiance regimes. Antioxidant enzyme activity of SOD and CAT and the MDA, GR and TP contents for the two species increased with increasing irradiance levels. Invasive *A. artemisiifolia* was able to scavenge oxygen radicals more efficiently at higher irradiance levels by enhancing CAT activity and GR and TP contents although leaf SOD activity was not greatly enhanced. This exotic species also maintained normal physiological functions when subjected to low irradiance, which might be attributed to the increase in POD activity with decreasing irradiance levels. The higher efficiency of adaptive responses of antioxidant enzymes may protect plants from irradiance-induced stress and may contribute to the invasiveness of *A. artemisiifolia* in subtropical and tropical regions.

Keywords: Abiotic stress, *Ambrosia artemisiifolia*, antioxidative enzymes, common ragweed, invasive plants, irradiance, lipid peroxidation, *Urena lobata*.

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Mots clés : *Ambrosia artemisiifolia*, enzymes antioxydants, flux énergétique, peroxydation lipidique, petite herbe à poux, plantes envahissantes, stress abiotique, *Urena lobata*.

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INTRODUCTION

The dramatic increase in exotic plant invasions has resulted in substantial damage to the structure, biodiversity and function of numerous ecosystems worldwide (Mack *et al.* 2000). Invasive plants generally exhibit greater stress tolerance, they can acclimate to a wider range of environmental conditions (Alpert *et al.* 2000; Feng *et al.* 2007), and are able to make more efficient use of fluctuating resources than native species (Davis *et al.* 2000). Light is the most important resource for plants and it influences both vegetative and reproductive growth (Chazdon and Pearcy 1986; Demmig-Adams and Adams 1992; Poorter 2001; Feng 2008). Invasive species appear to be better suited than native species to capture and utilize light resources, particularly in high light environments such as frequently disturbed habitats (Williams and Black 1994; Pattison *et al.* 1998). Increased light capture and utilization efficiency of successful invaders have been attributed to their morphological and physiological traits. For instance, Pattison *et al.* (1998) found that invasive species are better able to adjust biomass allocation and photosynthetic capacities across different light regimes, yielding higher growth rates than native species. However, determining the specific mechanisms by which invasive plants acclimate to varying irradiance levels and possibly benefit from these adaptations is currently understudied.

Like other abiotic stresses, high irradiance levels may induce the production of a variety of active oxygen species (AOS) such as superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl (OH^-) radicals (McCord 2000). Plants subjected to exposure to activated forms of oxygen and accumulation of free radicals may sustain damage to membranes and experience buildup of lipid peroxides (Smirnoff 1993; Hernández *et al.* 2000; Urquiaga and Leighton 2000). Cell membrane stability has been used to differentiate stress tolerant and stress susceptible varieties within crop plants (e.g. wheat) (Blum and Ebercon 1981; Bajji *et al.* 2002). In some cases, higher membrane stability has been correlated with increased performance or adaptation to stressful environmental conditions. Increases in the activity of antioxidant systems may help plants acclimate to irradiance stress (Grace and Logan 1996; Monk *et al.* 1989; Logan *et al.* 2006; Cheeseman 2007). Several studies have shown that plants produce antioxidant enzymes such as superoxide dismutase (SOD), peroxidase (POD), ascorbate peroxidase (APOX), catalase (CAT) and glutathione reductase (GR) to scavenge active oxygen species (Mishra *et al.* 1993; Ali *et al.* 2005; Kartashov *et al.* 2008), while relatively few studies have investigated the role of antioxidation in acclimation to irradiance of invasive plants.

Ambrosia artemisiifolia L. (common ragweed – Asteraceae), an herb species native to North America, was introduced into China in the 1930s and has extended its range from northern to southern China during the past three decades (Wan and Wang 1990; Qi *et al.* 2011). The latitudinal distribution of *A. artemisiifolia* in China ranges from 49°N to 24°N, covering more than 20 provinces and municipalities. Climate in northern regions is characterized as north-temperate, with mean annual air temperatures of 6-10°C and average annual precipitation of 530–680 mm, to subtropical in southern regions with mean annual air temperatures of 18–21°C and average annual precipitation of 1300–2400 mm. Moreover, about 54% of the annual solar radiation (4965 MJm^{-2}) received in northern regions occurs at temperatures above 10°C whereas in southern regions, 90% of annual solar radiation (4683 MJm^{-2}) occurs at these temperatures (climate data available from China Meteorological Data Sharing Service System, http://cdc.cma.gov.cn/index.jsp). With its rapid growth, plasticity and high reproductive output, *A. artemisiifolia* has successfully colonized disturbed habitats such as croplands and open, semi-natural and natural areas in Shaoguan, Jieyang and Huadu in the Guangdong province of southern China in recent years (Zeng *et al.* 2009). The presence of a wide range of spatially or temporally heterogeneous light environments in colonized regions provides an opportunity to assess the ecophysiological traits of *A. artemisiifolia* that may contribute to its invasive success, especially in the warmer subtropical regions of China. In a companion study, Zhong *et al.* (unpublished) observed enhanced morphological and photosynthetic physiological acclimation of *A. artemisiifolia* plants to a range of light regimes, particularly high irradiance levels. These adaptations were suggested to favour the recent invasive success of this exotic species in subtropical regions of China.

In this paper, we report on a comparative study of the exotic invader *A. artemisiifolia* and a native co-occurring species, *Urena lobata* L. (Caesar weed – Malvaceae) to determine their leaf antioxidant enzyme activity responses and malondialdehyde (MDA) content under different irradiance levels. These antioxidant enzymes and products play a key role in the scavenging of active oxygen species that damage the integrity and stability of plant cell membranes. We also evaluated differences between the two species in their antioxidant defense system, lipid peroxidation and irradiance capture efficiency. We used the native *U. lobata* in this study because it frequently co-occurs with *A. artemisiifolia* in disturbed, open habitats in southern China. We hypothesized that *A. artemisiifolia* exhibits a higher antioxidative capacity than *U. lobata*, and may receive increased protection from reactive oxygen species by increasing antioxidant enzyme activity, especially under extreme irradiance levels.

MATERIALS AND METHODS

Seed collection

Seeds of *A. artemisiifolia* and *U. lobata* were collected in late October 2009 from plants in several old fields that had at least 50% cover of *A. artemisiifolia*. The fields were located in the city of Shaoguan, province of Guangdong, China (24.8°N, 113.6°E). Typical climatological data for this climate region were described previously. Fully developed and average-sized seeds of the two species were selected and sown separately in seedbeds in a glasshouse on 6 March 2010. When seedlings of the two species were approximately 15 cm tall, uniform-sized seedlings were transplanted singly into clay pots (20 × 16 × 19 cm) that were filled with red lateritic soil

collected from the 0 to 20 cm soil layer of an abandoned field near the experimental station. Seedlings were grown at 30% irradiance for 25 d. Shade houses (4 × 4 × 3 m each) were constructed and irradiance levels were created by covering the shade houses with different layers of black nylon shade netting, including no netting to allow 100% irradiance. The lower 30 cm of each shade house remained open to facilitate airflow and to maintain humidity and temperature levels as similar as possible between the shade houses. The relative irradiance in each shade house was estimated by comparing the integrated photosynthetic photon flux density (PPFD) in a shade house during a clear day with that in a shade house with no netting.

On 14 May 2010, when both *A. artemisiifolia* and *U. lobata* were in their branching stage, 30 plants from the two target species (15 individuals per species) were randomly selected and subjected to one of four irradiance treatments: (i) dense shade, (ii) low irradiance, (iii) medium irradiance, and (iv) full irradiance, corresponding to 10%, 30%, 55% and 100% irradiance, respectively. Seedlings were watered daily and a compound fertilizer ($N:P_2O_5:K_2O$ = 15:15:15%, Batian Eco-Engineering Company, Shenzhen, China) was applied at a rate of 2 g pot^{-1} monthly. On 12 July 2010, the two species were in their late branching stage, although several individuals had initiated flowering. Five to eight fully expanded leaves of *A. artemisiifolia* and *U. lobata* individuals located at similar stem heights from the soil surface were collected from each treatment between 09:00 and 11:00 and subjected to enzymatic activity, protein and MDA content assays. Four replicates were used for each treatment. Leaf samples were rapidly fixed in liquid nitrogen, and stored at -70°C until the start of the assay.

Measurements

For enzyme extracts, 1.0 g of fresh leaf samples were ground to a fine powder with liquid nitrogen and extracted with 50 mM phosphate buffer (pH 7.0). The extracts were centrifuged at 4°C for 30 min at 12 000×g and the resulting supernatants were collected and used for enzymatic activity, protein and MDA content assays.

The activity of SOD was determined by monitoring its ability to inhibit the photochemical reduction of nitroblue tetrazolium (NBT) at 560 nm (Beauchamp and Fridovich 1971). One unit of SOD was defined as the amount of enzyme necessary to inhibit the reduction of NBT by 50%. CAT and POD activity was determined using the methods described in Chance and Maehly (1955). To measure CAT activity, a decrease in absorbance at 240 nm following the decomposition of H_2O_2 was recorded, while for POD activity, the oxidation of guaiacol was recorded by the increase in absorbance at 470 nm. One unit of CAT or POD activity was defined as an absorbance change of 0.01 unit per min.

Lipid peroxidation was measured by the amount of MDA detected, a product of unsaturated fatty acid peroxidation. The MDA content was determined based on the thiobarbituric acid (TBA) reaction as described by Peever and Higgins (1989) with modification and measurements corrected for non-specific turbidity by substracting the absorbance at 532 nm. The concentration of MDA was calculated by using an extinction coefficient of 155 mM^{-1} cm^{-1} (Heath and Packer 1968). Free proline (Pro) content was determined spectrophotometrically at 520 nm following the method of Bates *et al.* (1973) and consisted in the colorimetric determination of the amount of coloured reaction product of proline with ninhydric acid. Proline concentration was determined using calibration curves from which the absorbance values recorded were converted to absolute amounts. GR content was determined by the reduction of 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB) at 412 nm according to Li *et al.* (2009). The tea polyphenol (TP) content was measured according to Zhang *et al.* (2009).

Statistical analysis

Differences in antioxidant enzyme activities and the contents of MDA, GR and TP among the four irradiance levels for the two species were analyzed with variance analysis (ANOVA), and a post hoc test (Duncan's multiple range test) was conducted at $P < 0.05$. Differences between *A. artemisiifolia* and *U. lobata* for a given irradiance level were detected using an independent sample t-test. Regression analyses were performed to examine the variations of leaf antioxidant system parameters under different levels of irradiance. All statistical analyses were performed using SPSS 17.0 (SPSS Inc., Chicago, IL, USA).

RESULTS

Irradiance had a profound effect on the leaf antioxidant enzyme system and the contents of MDA, GR and TP, while species influenced the antioxidant enzyme system except for SOD. Irradiance accounted for at least 85% of the overall variance for measured antioxidant enzyme system parameters except for SOD. There were also significant interactions between irradiance and species except for MDA and SOD contents (Table 1). There were similar trends between *A. artemisiifolia* and *U. lobata* in terms of SOD activity in response to different irradiance regimes (Fig. 1A). With increasing irradiance levels, SOD activity in both species increased gradually (Y_{SOD} = 40.465 – 2.534X, R^2 = 0.535, P = 0.001; Y'_{SOD} = 42.863 – 2.539X, R^2 = 0.571, P = 0.021, where Y_{SOD} and Y'_{SOD} represent SOD activity of *A. artemisiifolia* and *U. lobata,* respectively, and X represents irradiance levels). Since SOD catalyzes superoxide free radical dismutation into H_2O_2 and O_2, the increase in activity might signify the production of O_2^- during leaf exposure to the irradiance treatments. SOD activity in full sunlight increased by about 1.5-fold and 1.3-fold for *A. artemisiifolia* and *U. lobata*, respectively, compared with leaves subjected to the 10% irradiance treatment. For *A. artemisiifolia*, SOD activity at the three highest irradiance levels of 30%, 55% and 100% was greater than at the 10% level, while for *U. lobata*, SOD activity at the 100% and 55% irradiance levels was higher than at the 30% and 10% irradiance levels. There was no difference

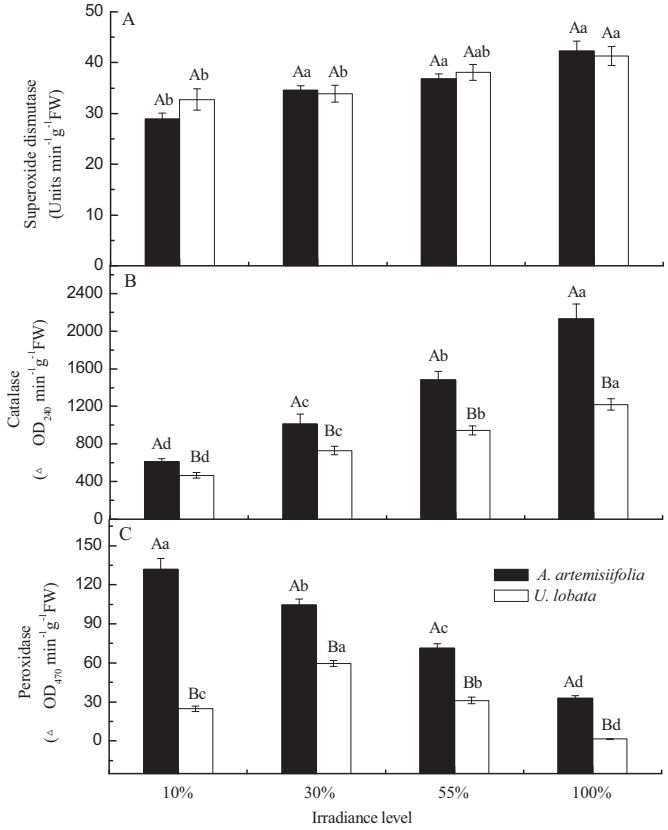

Figure 1. Superoxide dismutase (SOD) (A), catalase (CAT) (B), and peroxidase (POD) (C) activity in leaves of *A. artemisiifolia* (solid bars) and *U. lobata* (clear bars) under four irradiance treatments: (i) dense shade, (ii) low irradiance, (iii) medium irradiance and (iv) full irradiance, corresponding to 10%, 30%, 55% and 100% irradiance, respectively. Values are mean ± SE (n = 4). Different uppercase and lowercase letters indicate significant differences ($P < 0.05$) between the two species at the same irradiance level, and differences among irradiance levels for the same species (Duncan's multiple range test), respectively.

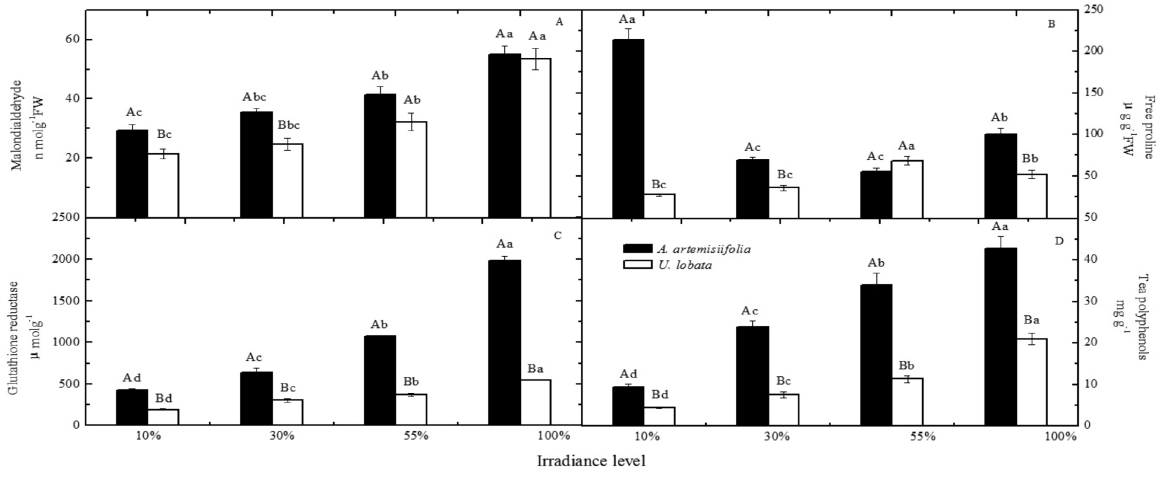

Figure 2. Contents of malondialdehyde (MDA) (A), free proline (Pro) (B), glutathione reductase (GR) (C), and tea polyphenols (TP) (D) in leaves of *A. artemisiifolia* (solid bars) and *U. lobata* (clear bars) under four irradiance treatments (10%, 30%, 55% and 100%). Values are mean ± SE (n = 4). Different uppercase and lowercase letters indicate significant differences ($P < 0.05$) between the two species at the same irradiance level and differences among irradiance levels for the same species (Duncan's multiple range test), respectively.

Table 1. Summary of nested ANOVA of the effects of target species (n = 2), irradiance level (n = 4) and their interactions on leaf antioxidant systems. Bold values indicate statistical significance ($P < 0.05$).

	Species			Irradiance			Interaction			
Antioxidant system	*F*	*P*	R_s^2	*F*	*P*	R_{ir}^2	*F*	*P*	Ri_n^2	R^2
MDA	15.310	**0.001**	0.389	47.818	**0.000**	0.857	1.468	0.248	0.155	0.872
Pro	196.821	**0.000**	0.891	43.8440	**0.000**	0.846	89.151	**0.000**	0.918	0.961
SOD	3.768	0.064	0.136	8.200	**0.001**	0.506	2.944	0.053	0.269	0.608
POD	429.675	**0.000**	0.947	108.782	**0.000**	0.931	37.324	**0.000**	0.823	0.973
CAT	183.338	**0.000**	0.884	52.955	**0.000**	0.869	20.129	**0.000**	0.716	0.944
GR	995.254	**0.000**	0.976	375.579	**0.000**	0.979	162.495	**0.000**	0.953	0.991
TP	203.205	**0.000**	0.894	75.621	**0.000**	0.904	13.542	**0.000**	0.629	0.951

F values, level of significance (*P*), and total R^2 of the model are shown. R_s^2, R_{ir}^2 and R_{in}^2 were calculated as the sum of squares of the effect (main effect of species and irradiance, and interactions in proportion to the total sum of squares, respectively).

between the two species under the four irradiance levels. The maximum value of SOD activity for *A. artemisiifolia* was 42.33 unit min^{-1} g^{-1} FW, which is about 2.4% higher than that for *U. lobata*.

Growth at higher irradiance levels was associated with an increase in CAT activity in both *A. artemisiifolia* ($Y_{CAT} = 2563.657 - 502.317X$, $R^2 = 0.890$, $P = 0.000$, where Y_{CAT} and X represent CAT activity and irradiance levels for *A. artemisiifolia*, respectively) and *U. lobata* ($Y'_{CAT} = 842.802 - 115.581X$, $R^2 = 0.733$, $P = 0.000$, where Y'_{CAT} represents CAT activity of *U. lobata*). CAT activity in *A. artemisiifolia* and *U. lobata* at the 100% irradiance level was 3.5 and 2.6 times that at the 10% irradiance level, respectively. CAT activity in *A. artemisiifolia* was higher than that in *U. lobata* under all irradiance levels. CAT activity under the four irradiance levels was 23.8% to 42.7% higher for *A. artemisiifolia* than for *U. lobata*, indicating that the exotic invasive herb was better able to adapt to light stress via enhanced CAT activity (Fig. 1B).

Ambrosia artemisiifolia exhibited an increase in POD activity with decreasing irradiance levels ($Y_{POD} = 2.523 + 33.018X$, $R^2 = 0.939$, $P = 0.000$, where Y_{POD} and X represent the POD activity and irradiance level in *A. artemisiifolia*, respectively), whereas *U. lobata* first displayed the same trend ($Y'_{POD} = 4.738 + 9.072X$, $R^2 = 0.306$, $P = 0.026$, where Y'_{POD} represents POD activity in *U. lobata*), but POD activity decreased at the lowest irradiance level of 10% (Fig. 1C). The lowest POD activity value for *A. artemisiifolia* was 32.78 unit min^{-1} g^{-1} FW, which is about 28 times that of *U. lobata*, although both species had the lowest POD activity under the 100% irradiance treatment. POD activity of *A. artemisiifolia* at the four irradiance levels was 53.2% to 72.9% higher than for *U. lobata. Ambrosia artemisiifolia* clearly had greater POD activity for leaves grown under all irradiance regimes, with a peak value of 131.76 unit min^{-1} g^{-1} FW observed at the 10% irradiance level.

Ambrosia artemisiifolia and *U. lobata* exhibited similar patterns in MDA content, which increased with increasing irradiance levels and peaked in the full sunlight treatment ($Y_{MDA} = 60.336 - 8.100X$, $R^2 = 0.794$, $P = 0.000$, $Y'_{MDA} = 59.002 - 10.400X$, $R^2 = 0.758$, $P = 0.000$, where Y_{MDA} and Y'_{MDA} represent the MDA activity of *A. artemisiifolia* and *U. lobata*, respectively, and X represents the irradiance level). When compared with *U. lobata*, the MDA content in *A. artemisiifolia* leaves under the four irradiance treatments was 2.2% to 26.9% higher than in *U. lobata* leaves, with the highest differences observed under the 10% and 30% irradiance regimes (Fig. 2A).

The Pro content in *A. artemisiifolia* leaves decreased from the 100% to 55% irradiance treatments, then increased with decreasing irradiance (Fig. 2B). The highest Pro content (214.2 ug g^{-1} FW) was observed at the 10% irradiance level, about 2.1 times that in the 100% irradiance treatment and 3.9 times that in the 55% irradiance treatment, thus indicating a higher Pro concentration due to irradiance stress. *Urena lobata* displayed a different trend from *A. artemisiifolia* in that Pro content increased in leaves subjected to the 55% irradiance level relative to full sunlight, then decreased at the 30% and 10% irradiance levels, reaching the lowest value of 27.0 ug g^{-1} FW in the 10% irradiance treatment. *Ambrosia artemisiifolia* had higher Pro activity than *U. lobata* in all but the 55% irradiance treatment.

GR content in both *A. artemisiifolia* and *U. lobata* increased with increasing irradiance levels (Fig. 2C). The difference between minimum and maximum GR levels in the two species remained at approximately 4.7- to 2.8-fold, respectively. *Ambrosia artemisiifolia* had a higher GR content than *U. lobata* under the four irradiance levels, but especially in the 100% irradiance treatment, where the GR content in *A. artemisiifolia* leaves was 3.7 times greater than that in *U. lobata* leaves. Higher GR levels for *A. artemisiifolia* indicate that the enzyme provided more antioxidant protection in response to irradiance in comparison to *U. lobata.* Relative to full sunlight, the 10% irradiance treatment caused a 78.9% and 64.4% reduction in GR content in *A. artemisiifolia* and *U. lobata* leaves, respectively.

Increasing irradiance led to increased levels in TP content in both *A. artemisiifolia* and *U. lobata* (Fig. 2D). Under the four irradiance regimes, the TP content in *A. artemisiifolia* leaves was greater than in *U. lobata* leaves.

DISCUSSION

The preference of the exotic invader *A. artemisiifolia* for open, sunny habitats suggests a sensitivity to light intensity. In low light environments, the growth and reproduction of this species is generally lower than in high light environments, although these plants can tolerate low light conditions and are capable of reproducing (Bassett and Crompton 1975). In high light, warm environments such as the subtropical regions of China, growth and fitness may be favoured because of increased photosynthetic rates and the ability of plants to reduce plant cell membrane damage from light-induced active oxygen species. Consistent with this characterization, we found that *A. artemisiifolia* plants were more efficient at acclimating to the varying irradiance conditions than the native co-occurring species *U. lobata*. Based on the quantity of antioxidant enzymes and on the contents measured, this greater ability of *A. artemisiifolia* to acclimate to a range of irradiance levels was most evident at the highest irradiance level (100%), although acclimation to the lowest (10%) irradiance level was also suggested in several cases.

As a widely distributed enzyme in higher plants, one of the main functions of POD is associated with its role in the cells' defense enzyme complex, which ensures the detoxification of activated oxygen forms. Higher POD levels have been observed in stress tolerant plant species, in which elevated POD activity protects the plants against oxidative damage (Scalet *et al.* 1995), whereas such activity has not been observed in sensitive plants (Peters *et al.* 1989). In our experiment, POD activity increased in both of our target species when subjected to low irradiance conditions, a response likely induced by increased levels of superoxide radicals resulting from a decline in SOD and CAT activity. This increase in POD activity was greater in *A. artemisiifolia* than in *U. lobata*, suggesting that *A. artemisiifolia* may be better able to survive and perform critical physiological functions, even at relatively low irradiance levels.

High SOD activity has been linked with stress tolerance in plants that survive treatments that enhance the production of O_2^- (Beauchamp and Fridovich 1971; Bowler *et al.* 1992). Increases in SOD activity has been observed in many plant species that have acclimated to temperature (Mishra *et al.* 1993), drought (Zhang and Kirkham 1994), or chilling stresses (Liu and Huang 2000). A similar higher SOD activity could be detected in our study in response to increasing irradiance. However, irradiance did not lead to a significant increase in SOD activity in *A. artemisiifolia* leaves relative to *U. lobata* leaves, suggesting that SOD may play a minor role in improving tolerance to irradiance stress in *A. artemisiifolia* plants. The comparatively lower SOD activity of *A. artemisiifolia* relative to *U. lobata* at the 55% and 10% irradiance levels does not imply that this exotic invasive species experienced more oxidative stress than the co-occurring native species since upon exposure to light, leaves of *A. artemisiifolia* exhibited higher activity of CAT enzymes as well as higher Pro, GR and TP contents than *U. lobata*, which points to specific non-enzymatic routes for the more effective conversion of O_2^- into H_2O_2 using antioxidants such as glutathione (GSH), ascorbate and phenol compounds (Asada 1999; Ali *et al.* 2005). Although the precise mechanism by which the activity of these enzymes is triggered remains to be determined, it might be assumed that CAT and POD enzymes as well as GR and TP contents are of equal importance in the detoxification of H_2O_2 in *A. artemisiifolia* leaves. This enhanced protection in *A. artemisiifolia* may reflect a more efficient antioxidative system given the higher CAT and POD enzyme activity and higher GR and TP contents.

Catalase, which is localized in the peroxisomes of higher plants and functionally protects cells against H_2O_2-mediated damage, may play a significant role in the defense against oxidative stress since H_2O_2 can readily diffuse across the membranes (Bowler *et al.* 1992). Increase in CAT activity supposedly is an adaptive trait that may help overcome photooxidative damage by reducing the toxic levels of hydrogen peroxide produced during cell metabolism (Grace and Logan 1996; Willekens *et al.* 1997). In our study, the greater CAT activity, especially at the highest irradiance levels measured in *A. artemisiifolia* leaves compared with *U. lobata* leaves, suggests a more effective scavenging mechanism to remove highly damaging H_2O_2 from cells.

The quantity of MDA produced during peroxidation of membrane lipids is often used as an indicator of free radical damage to cell membranes under stress conditions (Premachandra *et al.* 1992). Therefore, irradiance stress may have resulted in more pronounced deleterious oxidative processes and lipid peroxidation in *A. artemisiifolia* than in *U. lobata*. However, accumulation rates of MDA with increasing irradiance in *A. artemisiifolia* were relatively lower than in *U. lobata*. From 10% irradiance to full sunlight, the MDA content in *A. artemisiifolia* increased 1.9-fold while that of *U. lobata* increased 2.5-fold, suggesting that *U. lobata* was more likely to suffer more oxidative damage under severe high light stress.

Consistent with other species experiencing environmental stresses (i.e. heat, drought, light), irradiance stress increased MDA content in both *A. artemisiifolia* and *U. lobata*, a response that may be attributable to the malfunction of the scavenging system, possibly leading to damage in the main cellular components (Monk *et al.* 1989). The relatively lower MDA accumulation rates under irradiance stress observed in *A. artemisiifolia* suggest slower lipid peroxidation development, thus providing more effective protection against oxidative damage since elevated CAT and POD activity as well as higher Pro, GR and TP concentrations were maintained compared with *U. lobata*.

Proline is generally assumed to serve as a carbon and nitrogen source for rapid recovery from stress and growth, a stabilizer for membranes and some macromolecules, and also a free radical scavenger (Jain *et al.* 2001). Accumulation of Pro under stress in many plant species has been correlated with stress tolerance, and its concentration has been shown to be generally higher in stress-tolerant plants (Ashraf and Foolad 2007). In our study, *A. artemisiifolia* accumulated about 7.9-fold and 1.9-fold more Pro than *U. lobata* at the 10% and 100% irradiance levels,

respectively, making it less likely for *A. artemisiifolia* to be severely affected by low light availability or intense sunflecks. Similar to CAT, GR could also play a role in the control of endogenous hydrogen peroxide through an oxidoreduction cycle involving glutathione and ascorbate (Smith *et al.* 1989). Reduction in GR activity probably results in a decrease in the levels of reduced glutathione, which is known to be an important factor in preventing oxidative injuries (Alscher 1989). Therefore, the higher levels of GR content with increasing irradiance found in the present study may be helpful in protecting membranes from peroxidation damage by trapping oxygen radicals, especially in *A. artemisiifolia*.

In summary, irradiance stress (high or low light) induced oxidative injury in *A. artemisiifolia* and *U. lobata* plants. Both species exhibited defensive mechanisms under the various stress treatments to protect themselves against free radicals as highlighted by the varying activity of antioxidant enzymes, and the varying MDA, Pro and TP contents. These enhanced defense mechanisms ensured the detoxification of reactive oxygen species such as O_2^- or H_2O_2 that may be generated in light-stressed leaves. However, *A. artemisiifolia* has developed a particularly more efficient antioxidant enzyme system relative to the native species *U. lobata* that increases the likelihood of survival and continued development of plants experiencing stressful light conditions such as deep shade or full sunlight. In a companion study, Zhong *et al.* (unpublished) found that *A. artemisiifolia* performs especially well at high irradiance levels, exhibiting greater plasticity in morphological and photosynthetic traits such as specific leaf area, leaf area ratio and root:shoot ratio than the co-occurring native *U. lobata*. We applied these results to the current study and obtained support for *A. artemisiifolia* displaying a greater antioxidative capacity in adapting to an irradiance gradient than a co-occurring native species. These ecophysiological adaptive traits in *A. artemisiifolia* have likely contributed to the the successful invasion and range expansion of this species in many parts of temperate Europe (Fumanal *et al.* 2008), and also in subtropical regions such as observed recently in southern China (Wang *et al.* 2009; Qi *et al.* 2011). This better understanding of how acclimation to irradiance in *A. artemisiifolia* may affect its colonizing and invasive success will facilitate the prediction of future invasions and range transformations in relation to the projected climate change and general evolutionary potential of invasive plants (Clements and DiTommaso 2011; Sang *et al.* 2011).

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