Physiological response of *Monimopetalum chinense* to light stress under habitat fragmentation

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ABSTRACT

To determine the effect of light stress under fragmental habitat on the physiology, this paper investigated the physiological responses of *Monimopetalum chinense* with different light intensities in the Xianyu Mountains (Anhui, China). The study showed that both weak and intense light brought about by habitat fragmentation could improve antioxidant enzymes activities, and promote electrical conductivity and malondialdehyde content of *M. chinense* leaves. However, too strong light could inhibit photosynthesis rates, superoxide dismutase, catalase, and ascorbate peroxidase activities. In addition, the characteristics of leaves were affected by light intensity at the fragmental habitat. Specifically, intense light was disadvantageous to photosynthesis and antioxidant enzymes of the species. Our results suggest that the biodiversity conservation of *M. chinense* is necessary, and that light intensity should be considered carefully when implementing conservation efforts.

Keywords: endangered; photosynthesis rate; antioxidant enzymes; electrical conductivity; lipid peroxidation

Human alteration of the global environment has triggered the 'sixth major extinction event' in the history of life, as well as it caused widespread changes in the abundance and distribution of organisms. Most forest ecosystems worldwide were fragmented by logging and other human disturbance, resulting in highly dissected landscape patterns (Turner 1996). One notable consequence of fragmentation is the increase in forest edge/interior ratio in remaining forest patches. An altered microclimatic environment around forest edges can directly influence plant reproduction, recruitment, and many others (Tomimatsu and Ohara 2004). Light is one of the main factors influencing the establishment and development of many forest species; it also plays a major role in the ecology of intrinsic species. However, excessive light energy can photoinhibit photosynthesis, leading to reduced photochemical efficiency and conductance, increased temperature of leaves, impairment of CO_2 assimilation rate, and photo-oxidative destruction of photosynthetic apparatus through the formation of reactive oxygen species (Valladares and Pearcy 1997).

Monimopetalum chinense, a woody vine member of the Celastraceae, grows in a limited mountain area on the board between Anhui, Jiangxi, and Hubei Provinces in China (Anonymous 1999). As for its capsular fruit and isomerous flowers, this new Celastraceous species belongs to the Celastroideae-Euonymeae; it is related closely to Euonymus, but with many differing aspects. As a monotypic genus, *Monimopetalum* contributes significantly in the evolutionary history of

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Celastraceae species (Sytsma et al. 2002). However, due to anthropogenic disturbance and environmental deterioration, its habitat has been destroyed and is fragmented continuously, causing the species to be classified as endangered in the official list of Chinese rare species. Several studies were carried out, focusing on the biological and ecological factors associated with *M. chinense* decline (Ren 1989, Xie and Tan 1998, Hao et al. 2008, Zhang et al. 2008). However, studies on its physiological response to microclimate change under habitat fragmentation were not extensive. In the present study, the physiological responses of *M. chinense* under fragmental habitat were investigated to determine the impact of light on physiology, as well as to explore the most favorable light range and endangering mechanisms.

MATERIALS AND METHODS

Site description. The study region (30°00'N, 117°18'E) is located in the Shangkeng Village at the Xianyu Mountains of Anhui Province. The two communities (A and B) of *M. chinense* are isolated by a highway and by some buildings constructed as recent as 10 years ago, with more than 50 m apart. Community A corresponds to an area of more than 5000 m^2; it is undisturbed and in good condition. Meanwhile, community B was seriously destroyed by logging in 1998, and continues suffering from human disturbance. The light intensity in community B is much higher than in community A.

Seven sites were selected for sampling, specifically those from the middle to the edge of communities A and B. Light intensity was measured in each site at 10:30 a.m. on July 15, 2007 and July 13, 2008. The mean values for the two years were 40 (site I), 150 (site II) and 230 (site III) μmol/m^2/s for community A, and 460 (site IV), 720 (site V), 1190 (site VI) and 1650 (site VII) μmol/m^2/s for community B. At each site, healthy and adult individuals with root collar diameters between 10 to 15 mm were selected for sampling. The third pairs of leaves on the two-year-old branches were used to determine all physiological parameters at the closest time as possible. Net photosynthesis rate and stomatal conductance were measured directly in-situ. Other parameters were sampled into an ice pot and measured in a temporary laboratory. This laboratory was built in the Shangkeng Village.

Analyses. Light intensity, photosynthetic rate, and stomatal conductance were measured with a CI-340 portable photosynthesis system (CID Inc., Washington, USA). The leaf areas were measured with a Li-Cor 3100 (Li-Cor, Lincoln, Nebraska, NE) area meter and then dried at 68°C for 72 h and weighed.

The estimation of lipid peroxidation (i.e., malondialdehyde or MDA) was conducted according to the method of Velikova et al. (2000). Leaf membrane permeability was expressed as percentage of total electrolyte leakage and measured by electrical conductivity (Sukumaran and Weiser 1972). Total chlorophyll content was estimated according to the equations proposed by Wellburn (1994). The activity of superoxide dismutase (SOD) (EC 1.15.1.1) was determined by the photochemical method described by Giannopolitis and Ries (1977). Catalase (CAT) (EC 1.11.1.6) activity was assayed in a reaction mixture containing the enzyme and 25 mmol/l H_2O_2. Next, H_2O_2 decomposition was conducted at 240 nm (Cakmak and Marschner 1992). Determination of ascorbate peroxidase (APX) (EC 1.11.1.11) activity was performed as described by Nakano and Asada (1981). Peroxidase (POD) (EC 1.11.1.7) activity was assayed using the method of Luck (1963). The measurements were made in five replicates.

Statistical analyses were carried out by one-way analysis of variance (ANOVA) using Student's *t*-test for the evaluation of significance of differences. A correlation analysis was applied to test the relationship of physiological characteristics in the SPSS software.

RESULTS

Leaf parameters in different habitats. Leaf parameters of *M. chinense* varied under different light intensities, and significant differences (P < 0.05) were observed (Table 1). With light intensity increase, the fresh weight per leaf area, water content per leaf area, and ratio of chlorophyll a/b first decreased, then finally increased. Chlorophyll content increased gradually with decrease in light intensity, but it was reduced when light intensity was too weak; the opposite was observed for fresh weight per leaf area and chlorophyll a/b. The stomatal conductance increased with light intensity at about 40–460 μmol/m^2/s, but decreased when it was over 460 μmol/m^2/s.

Photosynthesis rate in different habitats. Net photosynthesis rate increased linearly with light intensity at about 40–460 μmol/m^2/s, but decreased rapidly when light intensity was over 460 μmol/m^2/s (Figure 1). Moreover, it was significantly (P < 0.05) enhanced by 641% from 40 to 460 μmol/m^2/s,

Table 1. The characteristics of *Monimopetalum chinense* leaves with different light intensities under fragmental habitats

Site	Light intensity ($\mu mol/m^2/s$)	Fresh weight per leaf area (mg/cm²)	Water content per leaf area (mg/cm²)	Stomatal conductance ($\mu mol\ H_2O/m^2/s$)	Content of chlorophyll (mg/g DW)	Chlorophyll a/b
I	40	13.49 ± 1.15^{a}	9.96 ± 0.52^{cde}	65.85 ± 7.36^{c}	2.63 ± 0.20^{a}	2.60 ± 0.14^{d}
II	150	13.97 ± 0.87^{a}	9.51 ± 0.38e	84.42 ± 12.92^{bc}	2.83 ± 0.19^{b}	2.75 ± 0.12^{cd}
III	230	14.18 ± 0.67^{a}	10.22 ± 0.47^{cde}	126.47 ± 19.20^{ab}	2.61 ± 0.15^{bc}	2.82 ± 0.09^{cd}
IV	460	14.11 ± 1.22^{a}	11.53 ± 0.44^{bcd}	145.87 ± 23.68^{a}	2.31 ± 0.06^{cd}	2.91 ± 0.15^{bcd}
V	720	15.35 ± 1.22^{ab}	11.77 ± 1.10^{bc}	133.84 ± 10.29^{a}	1.95 ± 0.21^{de}	3.01 ± 0.09^{bc}
VI	1190	16.14 ± 0.84^{ab}	12.50 ± 0.53^{ab}	105.00 ± 17.99^{abc}	1.70 ± 0.12^{ef}	3.22 ± 0.15^{ab}
VII	1650	17.61 ± 1.25^{b}	13.87 ± 0.77^{a}	84.29 ± 7.73^{bc}	1.24 ± 0.11^{f}	3.51 ± 0.08^{a}

All values are means ± S.E. (n = 5). Different letters after S.E. indicate a significant difference ($P < 0.05$)

but significantly ($P < 0.05$) declined by 46% from 460 to 1650 $\mu mol/m^2/s$, indicating that intensive light is not favorable for the photosynthesis rate of *M. chinense*.

Electrical conductivity and MDA content. Electrical conductivity proportionally increased with the increase in light intensity; it was lowest at 40 $\mu mol/m^2/s$ and highest at 1650 $\mu mol/m^2/s$ (Figure2). MDA content decreased gradually with the decline in light intensity. These results indicate that the peroxidation of *M. chinense* membrane lipids is intensified when exposed to intense light. Moreover, correlation analysis also showed that there was a very significant positive correlation between electrical conductivity and MDA content ($r = 0.75^*$, $n = 8$).

Antioxidant enzymes. SOD (Figure 3a) increased to a maximum at 460 $\mu mol/m^2/s$ and then decreased. The trends of CAT activity (Figure 3b) and APX activity (Figure 3c) were quite similar, with activities first decreasing, then increasing, and finally decreasing with light intensity increase. Correlation analysis showed that there was a significant positive correlation among the two enzymes ($P < 0.01$). However, POD presented a different trend (Figure 3d). When light intensity was lower than 460 $\mu mol/m^2/s$, antioxidant enzymes activities declined first and then increased; this indicates that both weak and intense lights can improve the metabolism of ROS. When light intensity was more than 460 $\mu mol/m^2/s$, the activities of SOD, CAT, and APX began to decrease, indicating that the light stress exceeded its tolerance. The result was similar to the related studies (Sofo et al. 2003).

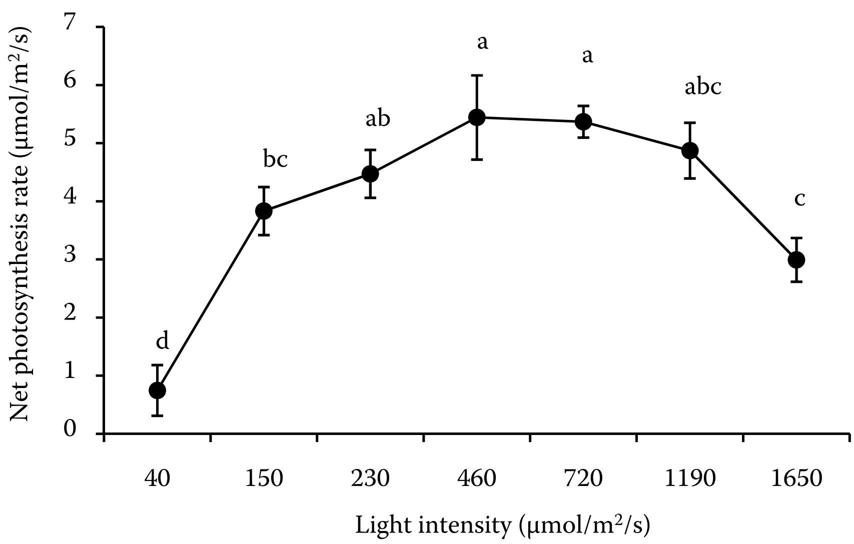

Figure 1. Net photosynthesis rate of *M. chinense* leaves with different light intensities under fragmental habitats. Data correspond to mean ± S.E. (n = 5). Different letters after S.E. indicate a significant difference ($P < 0.05$)

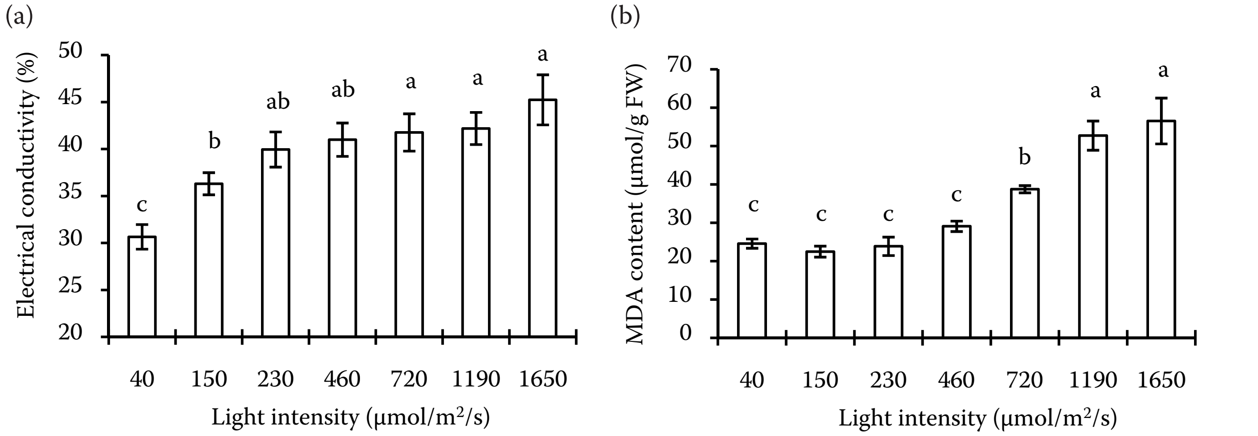

Figure 2. Electrical conductivity (a) and MDA content (b) of *M. chinense* leaves under different light intensity in fragmental habitats. Data correspond to mean ± S.E. (n = 5). Different letters above the bars indicate a significant difference (P < 0.05)

DISCUSSION

Light is the only energy source required for photosynthesis in higher plants; it is a basic requirement for plant growth and development. However, light can also harm the so-called sun plants if it is too weak to satisfy photosynthesis requirements, or if the light energy absorbed by leaves of shade plants exceeds the capability of photosynthetic apparatus (Valladares and Pearcy 1997). Our results revealed that *M. chinense* demonstrated similar characteristics as shade plants (Niinemets et al. 1998), indicating that this species can endure shady habitat to a certain extent. Studies on its stem and leaf anatomy proved that this species prefer shaded habitats (Ren 1989). The results

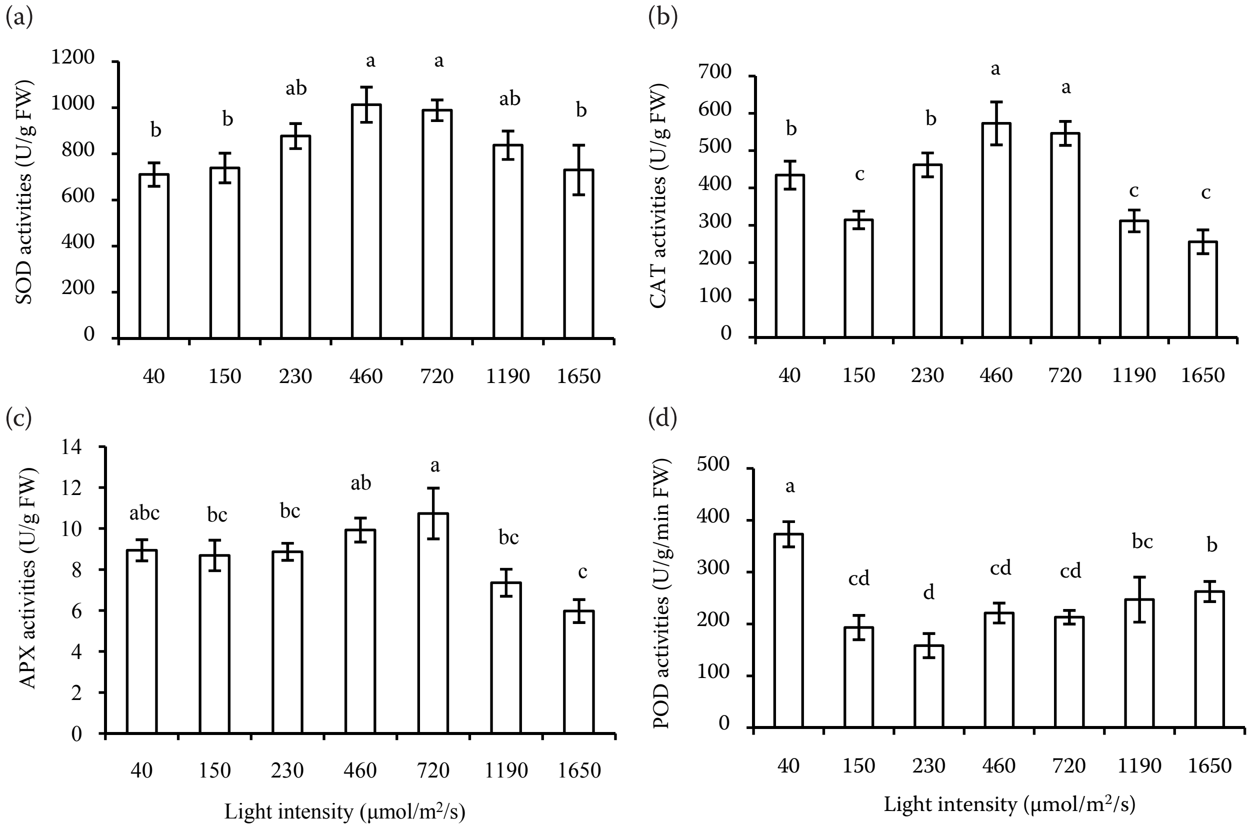

Figure 3. Antioxidant enzymes, SOD (a), CAT (b), APX (c), and POD (d) of *M. chinense* leaves with different light intensities under fragmental habitats. Data correspond to mean ± S.E. (n = 5). Different letters above the bars indicate a significant difference (P < 0.05)

presented by Xie and Zhang (1999) showed that this species is suitable for habitats with medium canopy density. Based on the analysis, *M. chinense* belongs to the shade-living plant group, but it cannot endure light that is too weak.

Intense light induces severe lipid peroxidation due to the removal of hydrogen by reactive oxygen species from unsaturated fatty acid, thus leading to lipid radical formation (Sofo et al. 2003). Dimerization and polymerization of proteins also occur, and these are considered as most damaging to the membranes (Bor et al. 2003). In relation, lipid peroxidation corresponds to the basic cell membrane reactive damage in cellular mechanisms. The electrical conductivity and MDA content under 1650 μmol/m^2/s increased by 48% and by 151%, respectively, compared with their lowest values. These indicate that lipid peroxidation in *M. chinense* leaves is very sensitive to changes in light intensity. In addition to carotenoids, antioxidative systems play significant roles in protecting plants from the negative effects of reactive oxygen species resulting from light energy excess (Demmig-Adams 1998). The antioxidant enzymes, SOD, CAT, POD, and APX, play vital roles in scavenging these destructive oxidant species. By catalyzing the detoxification of $O_2^-\bullet$ to O_2 and H_2O_2, SOD can prevent $O_2^-\bullet$ -driven cell damage; meanwhile, CAT, POD, and APX can remove H_2O_2 (Cakmak and Marschner 1992, Aravind and Prasad 2004). Our current study also showed that SOD, CAT, POD, and APX activities changed with light intensity, indicating a close correlation between light intensity and the physiological behavior of the species.

In general, low levels of stress can lead to an increase of antioxidant enzymes activities and remove $O_2^-\bullet$ (Yao et al. 2008). However, when accumulation of reactive oxygen species under more severe stress condition exceeds the removal capacity of the antioxidant system, oxidative damage occurs, including peroxidation of membrane lipids, destruction of photosynthetic and inactivation pigments, and inactivation of photosynthetic enzymes (Foyer et al. 1994, Sofo et al. 2003). Nevertheless, light intensity above 460 μmol/m^2/s might lead to an imbalance between antioxidant defense and amount of active oxygen species, thereby resulting in more severe stress in the physiology of *M. chinense*.

M. chinense prefers to live at the foot or slope of hills of low latitudes, making it vulnerable to the effects of human activities. With excessive tree cutting and serious destruction of the eco-environment, more and more *M. chinense* populations are now located in fragmental habitats. Small population size, isolation of populations from each other, and habitat light disturbance could be the three key factors associated with increased risks of species extinction. Based on this study, *M. chinense* has adapted to low light intensity, as high levels of light intensity is disadvantageous to its photosynthesis and antioxidant enzymes. The change in solar radiation under habitat fragmentation has influenced the physiology and ecology of *M. chinense*. According to the reports gathered from our field work, the seeds and seedlings of *M. chinense* are seldom found in fragmented habitats.

Based on the above analysis, the present work demonstrated that the change in light intensity under fragmental habitat could markedly affect the leaves characteristics, photosynthesis rates, and antioxidant enzymes activities of *M. chinense*. Although adaptable to shady habitat, both intense and too weak light are disadvantageous to this species. Therefore, light intensity should be considered carefully when implementing biodiversity conservation for *M. chinense*, especially during transplant conservation. The results obtained in this study can improve our knowledge on the effects of habitat fragmentation on *M. chinense* and impending efforts to implement biodiversity conservation.

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