BRIEF COMMUNICATION

Response of photosynthesis and chlorophyll fluorescence quenching to leaf dichotocarpism in *Ligustrum vicaryi,* an ornamental herb

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Abstract

Net photosynthetic rate of yellow upper leaves (UL) of *Ligustrum vicaryi* was slightly, but not significantly higher than that of green lower leaves (LL). Diurnally, maximum photochemical efficiency of photosystem 2, PS2 (F_v/F_m) of LL did not significantly decline but the UL showed fairly great daily variations. Yield of PS2 of UL showed an enantiomorphous variation to the photosynthetically active radiation and was significantly lower than in the LL. Unlike F_v/F_m, the efficiency of energy conversion in PS2 and both non-photosynthetic and photosynthetic quenching did not differ in UL and LL. Significant differences between UL and LL were found in contents of chlorophyll (Chl) *a*, *b*, and carotenoids (Car) and ratios of Chl *a*/*b*, Chl *b*/Chl (*a*+*b*), and Car/Chl (*a*+*b*). Leaf colour dichotocarpism in *L. vicaryi* was mainly caused by different photon utilization; sunflecks affected the LL.

Additional key words: carotenoids; chlorophyll; CO_2 uptake; energy utilization; photoprotection; sunflecks; thermal dissipation.

Plants have developed a number of strategies to balance the captured photon energy with photosynthesis, and protect photosynthetic apparatus against photoinhibition or photodamage (Anderson *et al.* 1997, Jiang *et al.* 2006). Diurnal trend in gas exchange is one of the indicators reflecting the ability of plants to maintain their photosynthetic apparatus in changing environment (Geiger and Servaites 1994, Mielke *et al.* 2003). Chlorophyll (Chl) fluorescence quenching analysis is a non-invasive, powerful, and reliable method to assess the changes in function of photosystem 2 (PS2) under different environments (Schreiber *et al.* 1994, Colom 2003, Mauchamp and Mèthy 2004). As a very sensitive intrinsic probe of photosynthesis, Chl fluorescence has been routinely used to monitor non-invasively the photosynthetic performance of plants. It checks the composition and organization of photosystems, the exciton energy transfer, the photochemistry, and the effects of various stresses on plants.

Plants in natural environments often experience considerable variations in accepted photosynthetically active radiation (PAR) caused by canopy structure, leaf age, and leaf architecture (Smith *et al.* 1989, Cui *et al.* 2006, Sobrado 2008). *Ligustrum vicaryi* (Oleaceae), a hybrid of Californian *L. ovalifolium* var. *aur-eomargi-natum* and *L. vulgale*, is widely used as landscape shrub for horticultural ornamentation. It possesses leaves of two different colours in the upper and lower position. The upper canopy develops relatively specific yellow even photo-bleached leaves; the lower ones tend to be green during the whole growth season due to architecture of canopy (dichotocarpism). 70−80 % of radiant energy is intercepted by the upper leaves (UL) and sunflecks are consequently the main photon source for the lower leaves (LL). In the differentiation process, high irradiance plays a key role in generating the two types of leaves. The variation in gas exchange, Chl fluorescence, and amounts of xanthophyll cycle components in different leaves reflects the different response to environmental changes. The objectives of this study were to find: (*1*) the different patterns in diurnal gas exchange and Chl *a* fluorescence parameters in UL and LL; (*2*) the physiological mechanisms leading to different leaf colour performance influenced by irradiation.

Received 10 June 2008, *accepted* 15 October 2008.
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Acknowledgments: The Program for Science and Technology Innovation Talents in Universities of Henan Province (No. 2008 HASTIT003) and the Natural Science Foundation of China (No. 30700078) supported this study. Thanks are given to Duan Jichuang and Ma Qingjun for their field help.

L. vicaryi, growing in nature accepts the full solar irradiation during the whole day. Net photosynthetic rate (P_N) was measured using portable photosynthesis system (*CIRAS-1, PP Systems*, Hitchin, UK). The measurements of Chl *a* fluorescence induction and its parameters were done by using a pulse amplitude-modulated fluorometer (*FMS-2*, *Hansatech*, Norfolk, UK) as described by Roháček and Barták (1999). The minimal (dark) (F_0) and maximal (F_m) fluorescence yield was obtained with weak modulated radiation (0.04 μmol m^{-2} s^{-1}), then with a 1-s pulse of saturating radiation (8 000 μmol m^{-2} s^{-1}). F_v/F_m is a measure of the maximum photochemical efficiency of PS2. Photochemical quenching (q_P) and non-photochemical quenching (NPQ) were calculated according to Schreiber *et al.* (1986) and Bilger and Björkman (1990), respectively (for review *see* Roháček 2002). The efficiency of energy conversion in PS2 (Φ_{PS2}) was calculated as $F_m' - F_s/F_m'$ (F_s = stationary fluorescence emission, F_m' = maximum fluorescence during irradiation) (Genty *et al.* 1989). Before fluorescence measurements, leaves were adapted to darkness for 20 min. Ten each healthy ULs and LLs of *L. vicaryi* were selected for both net photosynthetic rate (P_N) and fluorescence measurements. Photosynthetic pigment contents were measured according to Arnon (1949).

The diurnal variations of P_N of UL and LL of *L. vicaryi* showed double-peak curve, midday depression was observed at 11:00 and 13:00 for LL and UL, respectively (Fig. 1*A*). There was a time lag of one hour between P_N and midday depression points. P_N of LL and UL reached maximum at 10:00 and 11:00, respectively. P_N of UL was slightly, but not significantly higher than that of LL (t = 0.889, df = 18, p=0.386) (Independent-Samples T Test, where t stands for "t test value", *df* represents "degree of freedom" and *P* indicates "possibility"; the same as below).

F_v/F_m exhibited different diurnal patterns in UL and LL (Fig. 1*B*). F_v/F_m of LL did not significantly decline diurnally and averaged at 0.901 throughout the day. However, F_v/F_m of UL declined from 08:00 to 14:00 and reached the lowest value of 0.546 at 14:00 after which it tended to recover to 90 % of the value measured at 08:00. A great difference was also found between F_v/F_m values of the UL and LL (t = −11.809, df = 18, p=0.000). Thus there was significant difference between UL and LL in their light-harvesting ability when exposed to different irradiances.

Φ_{PS2} showed different diurnal patterns in UL and LL: in LL it exhibited irregular variation during a day, but in UL showed an enantiomorphous variation to the PAR, *i.e.* Φ_{PS2} declined from 08:00 to 12:00 and reached the lowest value of 0.230 (Fig. 1*B*). After 12:00, Φ_{PS2} tended to recover to the initial level of 08:00. Despite irregular variation during a day, Φ_{PS2} of LL leaves was significantly higher than that of the UL (t = −2.816, df = 18, p=0.011).

Unlike F_v/F_m and Φ_{PS2}, NPQ of UL and LL of *L. vicaryi* showed a similar daily variation curve, *i.e.* NPQ gradually declined from 08:00 to 17:00 and reached the lowest values of 0.234 (UL) and 0.252 (LL), respectively (Fig. 1*C*). NPQ almost decreased by about 90 % for UL and LL. No difference was observed between NPQ values of the UL and LL (t = 1.366, df = 18, p=0.189). No significant differences were found in q_P between UL and LL (t = 0.040, df = 18, p=0.969). Electron transport rate (ETR = Φ_{PS2}×PAR×0.84×0.5) of UL almost remained unchanged with slight increase at noon, however, ETR of LL showed apparent diurnal change and the maximum occurred at about 13:00 (Fig. 1*D*). Significant difference in ETR were observed between UL and LL (t = −2.139, df = 18, p=0.046).

Contents Chl *a*, Chl *b*, and Car (Table 1) differed between the UL and LL of *L. vicaryi* (Chl *a*: t = −31.681, df = 10, p=0.000; Chl *b*: t = −26.526, df = 10, p=0.000; Car: t = −20.608, df = 10, p=0.000). The contents of Chl *a* and Chl *b* of LL were 4.84 or 5.66 times those of UL, respectively, however, content of Car was only 0.68 times that of UL. Ratios of Chl *a/b* and Car/Chl of UL were higher than those of LL (t = −28.527, df =10, p=0.000; t = 24.997, df = 10, p=0.000), however, ratios of Chl *b*/Chl (*a*+*b*) were lower (t = 17.421, df = 10, p = 0.000).

Table 1. Differences in contents of chlorophyll (Chl) and carotenoids (Car) [mg m^{-2}] and their ratios, water content [%], and dry mass [g m^{-2}] of upper yellow (UL) and lower green (LL) leaves of *L. vicaryi*. n = 6. The values are expressed as mean ± SD.

	UL	LL
Chl *a*	4.77±0.51	23.10±2.42
Chl *b*	0.89±0.15	5.05±0.90
Chl (*a*+*b*)	5.66±0.56	28.15±3.24
Chl *a/b*	5.60±1.42	4.70±0.48
Car	7.23±0.43	4.89±0.73
Car/Chl (*a*+*b*)	1.31±0.18	0.18±0.04
Chl *b*/Chl (*a*+*b*)	0.16±0.04	0.18±0.01
Water content	79.91±0.63	82.12±0.75
Dry mass	35.04±0.50	26.29±0.81

Despite photosynthetic midday depression in UL and LL of *L. vicaryi*, no significant difference was detected between them (t = 0.889, df = 18, p=0.386). The existing minor difference did not confirm our expectation that UL possess lower P_N than LL, because of their lower contents of photosynthetic pigments and exposure to higher irradiance (Table 1). This paradox enlightens that there must be another mechanism to counteract these disadvantages. We found a higher ratio of dry mass [mg] to area [cm^2] in UL (Table 1), which serves as protective mechanism to prevent unnecessary solar radiation from penetrating into the interior of leaves. We also observed higher ratios of Chl *b*/Chl(*a*+*b*) and Car/Chl(*a*+*b*) in UL than LL (t = 17.421, df = 10, p=0.000; t = 24.997, df = 10, p=0.000) (Table 1). Chl *b* is important for energy distribution between the two photosystems, especially under high solar irradiation. Furthermore, Car as a non-enzymatic protective system dissipate excessive solar

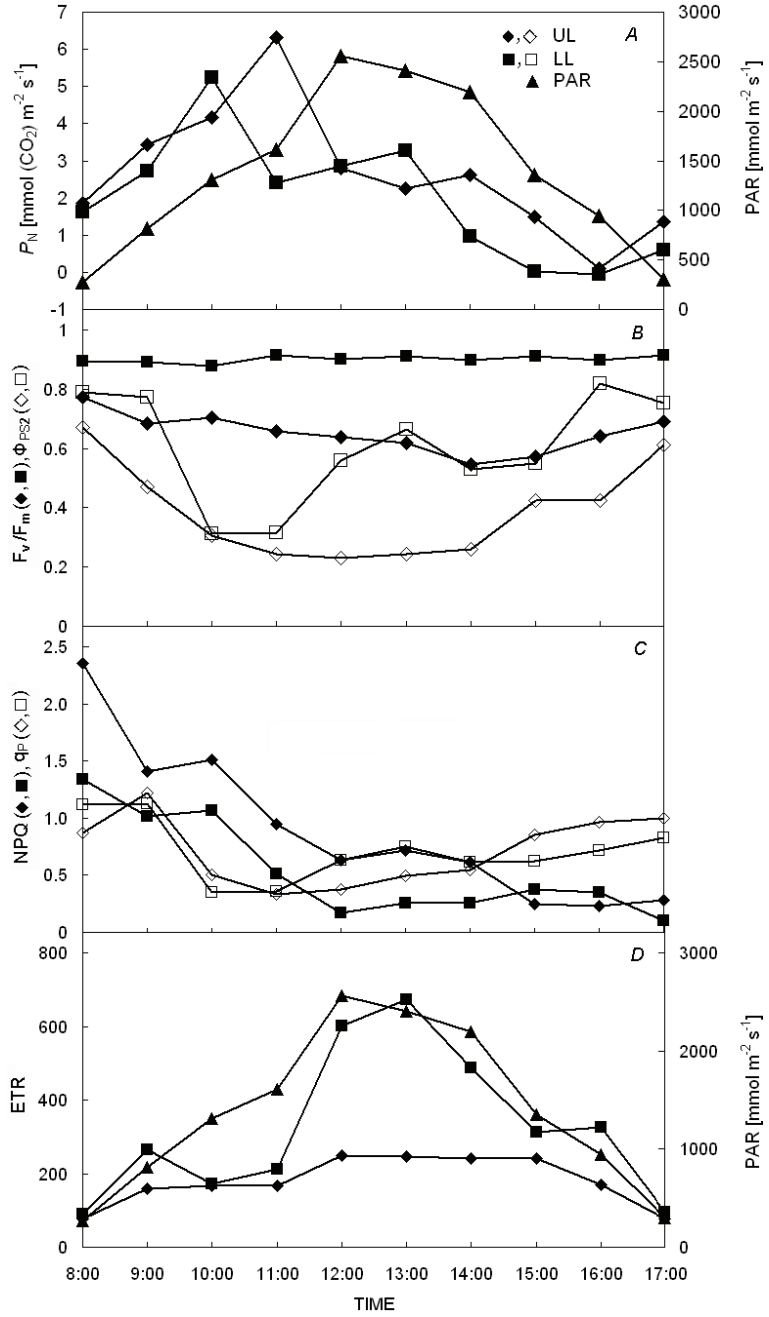

Fig. 1. Diurnal changes in (*A*) net photosynthetic rate (P_N) and photosynthetically active radiation (PAR), (*B*) F_v/F_m and Φ_{PS2}, (*C*) non-photochemical (NPQ) and photochemical (q_P) quenching, and (*D*) electron transport rate (ETR) in upper (UL) and lower (LL) leaves of *L. vicaryi*. Each point represents the mean of ten replicates, n=10.

energy. As illustrated in Fig. 1*D*, significant difference was detected in ETR between UL and LL which indicated that photorespiration might have contributed to the discrepancy between P_N and ETR.

Sudden decrease of PS2 effectiveness (Φ_{PS2}) in UL (Fig. 1*B*) confirmed our previous hypothesis that photosynthesis of UL of *L. vicaryi* may be inhibited under long-term high irradiation. Photoinactivation is inevitable in PS2 reaction centres, even under weak irradiation (see decline of Φ_{PS2} in LL; Fig. 1*B*). Significant differences in change of F_0 was observed in both UL and LL (2.82±0.85 and 39.82±5.49, $n = 29$, $p<0.001$), indicating different abilities to adapt to high irradiance. This could be partly explained by high irradiation-induced disorders in chloroplast ultrastructure. In the swollen and highly disorganized thylakoids the architecture of photosynthetic units and membrane-bound electron transport processes may be disturbed. There is evidence that leaf structure may also affect the primary fluorescence parameters even when F_v/F_m shows little or no change (Araus and Hogan 1994).

Our results demonstrated that both q_P and NPQ fluctuated simultaneously at all dynamic frequencies (Fig. 1*C*), which indicated quick redistribution of absorbed energy among different pathways, especially in UL of *L. vicaryi*. The decrease of NPQ to 0.4–0.5 reflects decreased

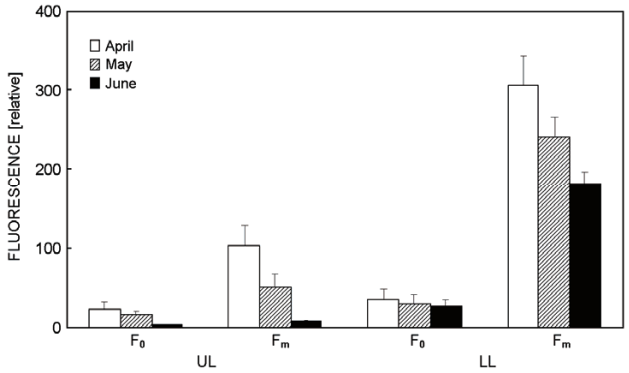

Fig. 2. Monthly changes in F_0 and F_m in upper (UL) and lower (LL) leaves of *L. vicaryi*. *n*=10. F_0 decreases dramatically without significant change in F_0/F_m due to simultaneously remarkable decline in F_m in UL.

thermal dissipation at the pigment level. In general terms, Φ_{PS2} decreases and NPQ increases in order to avoid photodamage. The decrease in NPQ, however, was not correlated with increase in F_v/F_m or Φ_{PS2}. Thus, most of F_v/F_m changes of UL observed in high irradiance were not fully explained by variation in NPQ. Disorder or degradation may have occurred in UL at high irradiance. However, F_v/F_m tended to recover from 14:00 and reached 90 % of the value at 08:00, which indicated reparation at lower irradiance. Therefore, NPQ based on xanthophyll cycle may not be the main mechanism for excessive energy dissipation in *L. vicaryi*. The slight change of F_v/F_m was recorded together with a much stronger monthly decrease of F_0 (2.82±0.85) in UL (Fig. 2), implying that photoprotection by F_0 quenching was the main cause of F_v/F_m reduction. We found that very little difference in ETR (*i.e.* at rates of CO_2 fixation) occurred at high irradiances. The excess of photons in UL may cause a down-regulation of PS2 in order to avoid over-reduction of primary electron acceptor Q_A and to reduce the load on the ETC.

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