ประสิทธิภาพการดูดซับแสงและกระบวนการโฟโตเคมีที่แตกต่างกันของไลเคน *Parmotrema tinctorum* ในแหล่งอาศัยที่มีแสงมากและน้อย

DIFFERENT QUANTUM EFFICIENCY AND PHOTOCHEMISTRY OF THE LICHEN *Parmotrema tinctorum* IN SUNNY AND SHADED HABITATS

ศรัทธารา หัตถีรัตน์ และ กัณฑรีย์ บุญประกอบ
Sattara Hattirat and Kansri Boonpragob

Department of Biology, Faculty of Science, Ramkhamhaeng University, Bangkapi, Bangkok 10240, Thailand; Fax: (662) 310-8395, e-mail address: sattarah@gmail.com

บทคัดย่อ: การวิเคราะห์ปริมาณคลอโรฟิลล์ของไลเคน *Parmotrema tinctorum* ที่เก็บมาจากแหล่งอาศัยธรรมชาติที่มีแสงมาก (เช่น กลางแจ้ง) และที่มีแสงน้อย (เช่น ใต้เรือนยอดในป่า) ในอุทยานแห่งชาติเขาใหญ่ พบว่าส่วนขอบโลบของแทลลัสที่เติบโตในที่แสงต่ำมีปริมาณคลอโรฟิลล์โดยรวม คลอโรฟิลล์ *เอ* และคลอโรฟิลล์ *บี* มากกว่าแทลลัสที่เติบโตในบริเวณที่ได้รับแสงสูงอย่างมีนัยยะสำคัญทางสถิติ ส่วนกลางแทลลัสจากบริเวณทั้งสองมีปริมาณคลอโรฟิลล์ไม่แตกต่างกัน เมื่อศึกษาประสิทธิภาพการรับแสงของคลอโรฟิลล์จากการปลดปล่อยแสงฟลูออเรสเซนท์ พบว่าแทลลัสในที่แสงน้อยตอบสนองต่อปริมาณแสงต่ำได้ดีกว่า ในขณะที่แทลลัสจากที่แสงมากตอบสนองต่อปริมาณแสงที่สูงกว่า แสดงให้เห็นว่า *P. tinctorum* มีการปรับตัวเพื่อให้สามารถอาศัยในแหล่งอาศัยธรรมชาติที่มีสภาพแสงแตกต่างกันมากได้

Abstract: The green algal lichen *Parmotrema tinctorum* was collected from shaded and sunny habitats from the secondary forest at Khao Yai National Park for analysis of chlorophyll contents and measurement of chlorophyll fluorescence. Chlorophyll analysis showed that the shade thalli had significantly higher amount of total chlorophyll, chl *a* and chl *b* than the thalli from sunny habitats. The lobe margins contain larger amount of chlorophyll than the inner areas of thalli. The inner parts of thalli have less variation of chlorophyll contents among samples. These results suggested that the lobe margins are the productive zones of photosynthetic carbon assimilation. Chlorophyll fluorescence parameter demonstrated that quantum yield ($\Delta F/F_m$) of the shade thalli had higher photochemical activity under low light intensity than the sun thalli. They also reacted immediately to brief low irradiance (giving low fluorescence yield), but reacted more slowly (giving high fluorescence yield—PSII becoming less efficient) under brief high irradiance. By contrast, the sun thalli reacted more readily to high irradiance. The study shows that *P. tinctorum* have different strategies and adaptive capacity in utilizing light energy in different microhabitats.

Introduction: In the tropical rain forest, light is a major limiting factor for production and distribution. Spatial competition for illumination is severe among photosynthetic organisms. Photosynthetic organisms that can utilize light energy efficiently overcome others in habitat competition. *Parmotrema tinctorum* (Despr. ex Nyl.) Hale is a common foliose lichen in Khao Yai National Park (KYNP) [2]. They grow both under shade and in bright sunlight, covering the bark of trees and rock surface. This lichen is one of the fastest growing lichens in KYNP [4], which was reported to consist of novel chemical products [3]. They have been transplanted in the natural habitats in order to increase biomass production for conservation and sustainable utilization [5]. However, physiology of the species is not well understood. The objective of this study is to observe adaptive strategies of the lichen *P. tinctorum* under different light regimes in order to understand the lichens' physiological basis of light utilization. The hypothesis of the study was that lichens in shaded habitats were more capable of capturing and utilizing light than lichens in sunny habitats by producing more chlorophylls.

Methodology:

Chlorophyll analysis

Six lichen samples (*Parmotrema tinctorum*) each from highly illuminated and shaded habitats (on both rock and bark substrates) were collected from Khao Yai National Park in June 2007. Extraction of chlorophylls was prepared using dimethyl sulfoxide (DMSO) [1]. A spectrophotometer (UV-VIS, Shimadzu) was used to measure the optical density of the solution at 665 and 648 nm. Total chlorophylls, chlorophyll *a* and *b* contents were determined according to [1].

Chlorophyll fluorescence

Measurements of lichen light responses were carried out in the laboratory by exposing the samples with a series of five-minute irradiance of 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1200, 1400 μmol $m^{-2}s^{-1}$. Three lichen thalli from each habitat, sun and shade, were placed in a control chamber in a hydrated state and maintained so throughout the measurements. Temperature was kept constant at 29-31 ^{o}C by an air cooling device. In addition, lichen rapid responses to different light intensities were measured by exposing the samples to a series of five-second irradiance of 100, 200, 300, 400, 600, 1200, 2200 μmol $m^{-2}s^{-1}$. Chlorophyll fluorescence was measured using a miniature pulse-amplitude modulated photosynthesis yield analyzer (MINI-PAM, Walz, Effeltrich, Germany) and accompanied leaf-clip holder. Data analysis was conducted with SigmaPlot software (Jandel Scientific, San Rafael, Calif.). Light intensity of lichens' natural habitats was measured from 6.00 am. - 6.00 pm. using LI-1400 (LI-COR).

Results, Discussion and Conclusion:

Chlorophyll analysis

Chlorophyll contents of lichens from three different habitats are presented in table 1.

Habitats and thallus areas	Total chl		Chl *a*		Chl *b*	
	Mean	±SD	Mean	±SD	Mean	±SD
Shade-marginal	4.033_a	0.40	3.086_d	0.32	0.947_g	0.23
Shade-central	1.745_b	0.50	0.961_e	0.30	0.594_h	0.16
Sun-marginal	2.383_c	0.41	1.898_f	0.34	0.485_i	0.09
Sun-central	1.390_b	0.22	0.938_e	0.24	0.452_{hi}	0.07

Table 1 Average chlorophyll contents (mg g^{-1} air dry weight) of the lichen *Parmotrema tinctorum* (n = 6) from shaded and sunny habitats. Samples were taken from the marginal and inner zones of thalli. The marginal zones of shade lichen thalli had the highest contents of chlorophyll *a*, *b* and total chlorophyll. There were small variations of chlorophyll contents in the inner areas of thalli.

Total chlorophyll contents from the thallus margins of shade lichens were significantly higher than those of sun lichens (t-test: $p < 0.001$). However, total chlorophyll contents from the central zones of both shade and sun thalli were not significantly different ($p > 0.5$). The average chlorophyll *a* contents in the marginal zones of shade thalli were approximately 63%

higher than those of sun thalli while the average chlorophyll *a* contents in the central zones of both shade and sun thalli were not significantly different ($p > 0.5$). Chlorophyll *b* in the marginal areas of shade thalli were about 60% higher than those in the inner areas. Chlorophyll *b* of the sun thalli did not differ significantly between marginal and inner areas of thalli ($p > 0.5$). In summary, the marginal zones of shade lichen thalli had the highest contents of chlorophyll *a*, *b* and total chlorophyll. This suggested that in lower light condition (Figure 1b), lichens produced higher amount of chlorophyll *a* and *b* at the lobe margins in order to capture less available sunlight.

There were only small variations in chlorophyll *a* and *b* in the inner areas of different *P. tinctorum* samples although the lichens grow in very different environmental conditions. Contrastingly, the average total chlorophyll, chlorophyll *a* and chlorophyll *b* contents at the marginal zones showed large variation not only between shade and sun thalli but also among different samples. The results suggested that marginal lobes are the active zones for photosynthetic carbon assimilation [6]. The lower chlorophyll contents at the central areas of the thalli were not likely the mere results of age or tissue degradation as the central zones maintain equal contents of chlorophyll *a* and *b* among samples of different sizes and ages.

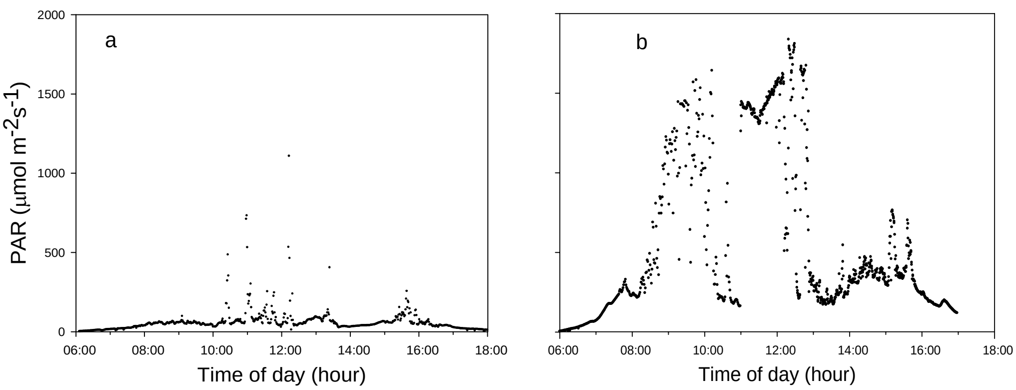

Figure 1 Daily light conditions (measured in June, 2007) at the locations where lichen samples, *P. tinctorum*, were collected: (a) an area under the shade of forest trees and (b) a highly illuminated site.

Chlorophyll fluorescence

The mean values of potential quantum yields (F_v/F_m) of shade lichens and sun lichens were 0.63 and 0.52 respectively, demonstrating higher efficiency of PSII photochemistry of the former. This is caused by the higher amounts of chlorophyll contents observed in the shade thalli. The actual quantum yields ($\Delta F/F_{m'}$) and electron transport rates (ETR) are presented in fig. 2a (shade lichens) and 2b (sun lichens).

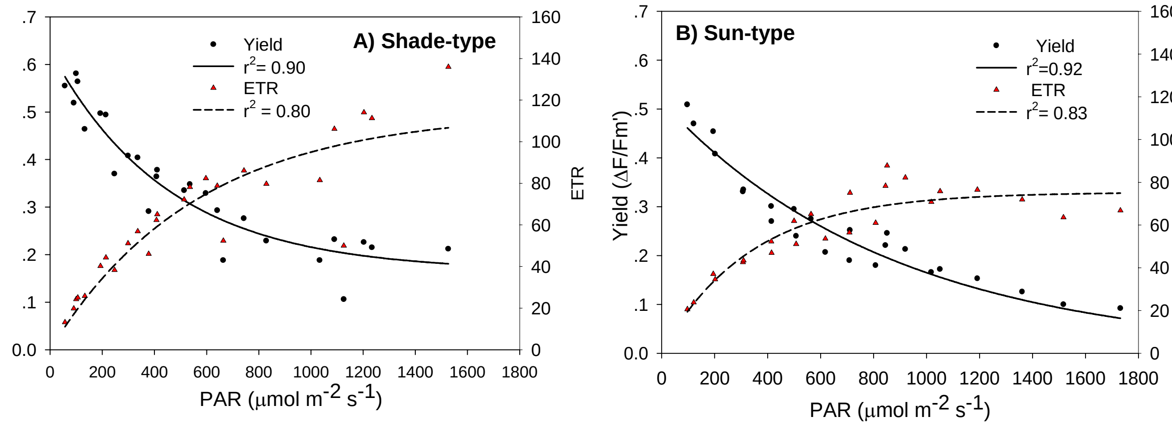

Figure 2 Light response curves of hydrated lichen *P. tinctorum* in control environment, showing ΔF/Fm' and ETR under five-minute exposures to 100-1800 µmol $m^{-2}s^{-1}$ irradiance: (a) shade-type lichens and (b) sun-type lichens; (n = 3)

Figure 2a shows that there was a rapid decrease of actual quantum yield (solid line) in accordance to a rapid increase of ETR (dotted line) in shade lichens exposed to 100-800 µmol $m^{-2}s^{-1}$ irradiance, indicating high efficiency of shade lichens' photochemical system under low to medium light intensity. However, at illuminations above 800 µmol $m^{-2}s^{-1}$, the quantum yields of shade lichens became more stable, indicating a light saturation of this lichen. In such conditions, the ETR of shade lichens remains substantial. This is probably due to excess PAR.

As shown in Figure 2b, the quantum yields of sun lichens declined more slowly and with smaller magnitudes than shade lichens under increasing light intensity. However, the values continue to decrease under high illuminations of up to 1600 µmol $m^{-2}s^{-1}$. This shows that sun lichens can continue their photosynthetic activity in high solar irradiance, provided that they are kept in hydrated state. This condition rarely occurs in nature because of vapor pressure deficit in the atmosphere, and intense radiation in the tropic.

These evidences show that the shade-type lichens had limited capacities to utilize high solar irradiance despite their high chlorophyll contents. The sun-type lichens acclimated to excessive light by producing lower but appropriate amount of chlorophylls to maintain photochemical activities in high solar energy. Lower amounts of harvesting pigments and high rates of photochemical conversion prevent damages or photoinhibition by high-light stress [7]. This strategy also avoids unnecessary allocation of photosynthetic products to construct excessive photosynthetic units.

Photosynthetic induction by short sunflecks has recently been the subjects of several studies on higher plants, ferns and aquatic algae [8]. Rapid Light Curve, which records with short illumination times of 5 seconds, allows insight into lichens' flexibility to rapid changes in light intensity, as shown in Figure 3.

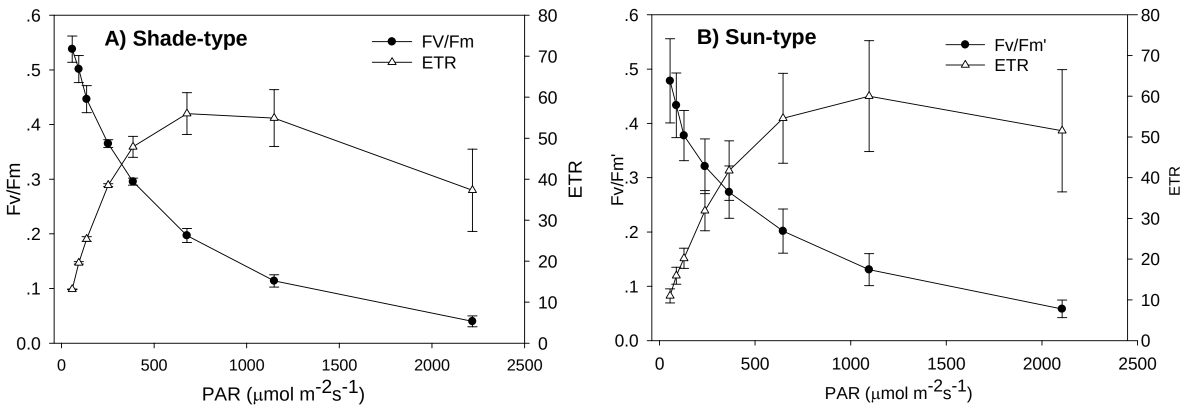

Figure 3 Rapid light response curve of hydrated lichen *P. tinctorum* in control environment, showing ΔF/Fm' and ETR under five-second exposures to 100-2200 μmol $m^{-2}s^{-1}$ irradiance: (a) shade lichens and (b) sun lichens; (n = 3)

Shade-type lichens, exposed to a series of lightflecks or brief irradiance, had lower ΔF/Fm' and higher ETR than sun-type lichens in low irradiance intensity (PAR 100-500 μmol $m^{-2}s^{-1}$), indicating that shade lichens responded immediately to low and brief illumination. However, at PAR more than 600 μmol $m^{-2}s^{-1}$, ETR of the shade lichens began to decline. ETR of sun lichens continued to rise up to 1,000 μmol $m^{-2}s^{-1}$. Thereafter, the reduction of ETR was observed. However, the ETR of the shade lichens declined more than that of the sun lichens at high light intensity.

This evidence underpins different strategies and adaptive capacity of *P. tinctorum* in utilizing light energy in different microhabitats. A fast photosynthetic response within the short duration of sunflecks, which are lower than 100 μmol $m^{-2}s^{-1}$ for most of the day (Figure 1a), optimize the light exploitation of shade-type lichens in a light-limited understory habitat [8].

In conclusion, the shade lichens had high chlorophyll contents but limited capacities to utilize high solar irradiance. They survived under low light intensity by producing large amount of chlorophylls so that brief and low irradiances, i.e. sun flecks (Fig 1b), are quickly captured. By contrast, the sun lichens survived high solar irradiance by producing just enough chlorophyll to capture excessive light energy. Therefore, they tend to have higher light saturation point, which enables them to take advantage of such condition for spatial competition.

Further studies on photochemical efficiency of *P. tinctorum* in shaded and sunny environments are needed in order to elucidate the adaptive capacity and acclimatization to light levels for highest production.

References:

1. K. Boonpragob, *Monitoring with Lichens—Monitoring Lichens*, 2002, 323-326.
2. K. Boonpragop, N. Homchantara, B. J. Coppins, P. M. McCarthy and P. A. Wolseley. *Botanical Journal of Scotland*, 1998, ***50***, 209-219.
3. W. Hongsachat, V. Poengsungnoen and P. Mongkolsuk, *32nd Congress on Science and Technology of Thailand*, 2006.
4. N. Osathanon, *Msc. Thesis*, Ramkhamhaeng University, Bangkok, 2002.

5. M. Pangpet and K. Boonpragob, *32nd Congress on Science and Technology of Thailand*, 2006.
6. M. Bartàk, J. Hàjek and J. Gloser, *Photosynthetica*, 2000, **38 (4)**, 531-537.
7. H. K. Lichtenthaler and S. Burkart, *Bulg. J. Plant. Physiol*, 1999, **25(3-4)**,3-16.
8. M. Lakatos, U. Rascher and B. Büdel, *New Phytologist*, 2006, **172**, 679-695.

Keywords: tropical lichens, light adaptation, marginal and central zones of thallus, PSII efficiency, chlorophyll contents, chlorophyll fluorescence