H_H0069: DAILY MICROCLIMATIC FACTOR OF THE HORIZONTALLY TRANSPLANTED HABITAT AND ITS INFLUENCES ON METABOLIC ACTIVITY AND GROWTH OF THE LICHEN PARMOTREMA TINCTORUM AT KHAO YAI NATIONAL PARK

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Abstract: Previous study found that growth of the transplanted lichens is the highest at the horizontal level comparing with other cardinal aspects. The aims of this study were to investigate diurnal fluctuation of microclimate, thallus water content, daily metabolic activity observed from chlorophyll fluorescence parameters, and growth of the lichen *Parmotrema tinctorum* transplanted on man-made substrate. The transplanted lichen were laid horizontally above the ground at Khao Yai National Park. After 3 months growth rate was 0.77 mm/month^{-1}. Photosynthesis, assessed from quantum yield of chlorophyll fluorescence, fluctuated with thallus water content and illumination. Daily metabolic activity could be divided into four periods. 1) Main photosynthetic activity in the morning, lasted for 5 hours, 2) dry thallus respiration during the day light, continued for 5 hours, 3) minor photosynthesis in the afternoon, occurred for 2 hours and 4) moist thallus respiration at night, persisted for 12 hours. NP under the control optimum condition of this lichen was 5.33 μmol CO_2 $m^{-2}s^{-1}$. This condition never occurred simultaneously in the field during this experiment. It implied that there is potential to modify the transplanted condition to meet the best condition for net photosynthesis in order to achieve higher biomass production of *P.tinctorum*. This lichen has great potential to be utilized in several aspects.

Introduction: Lichens are poikilohydric organisms, thallus water content entirely depended on atmospheric moisture. Wet and dry periods of thallus during the day affects photosynthetic capacity, growth and survival of lichen in the nature.[1] Chlorophyll fluorescence parameter, apparent quantum efficiency of PSII, fluctuates between the optimal value and zero depending on incident light and moisture status of the lichen. If lichen is dry and photosynthetically inactive, ΦPSII is zero. Thus, ΦPSII is an important indicator of photosynthetic active period under field conditions.[1,2,3]

Santanoo and Boonpragob (2013)reported that transplantation the lichen *P. tinctorum* on man-made substrate had the highest growth rate on the horizontal comparing with other four cardinal aspects.[4] This study investigated the influence of microclimate on photosynthetic activity in the field assessed by ΦPSII and ETR parameter of chlorophyll fluorescence, and evaluated its proportion with the control optimum condition. The information world be utmost important for enhance biomass production for sustainable utilization of this lichen.

Methodology:

Study area and lichen material

The transplanted station was setup at the training center 2, Khao Yai National Park, Thailand. Lichen transplantation began in March2014 by using 120small thalli, 2-3 cm^2, of *P.*

tinctorum collected form secondary forest. Each thallus was tied by nylon line on 5x5 cm^2 plastic net (Figure 1a.) that attached over another layer of plastic meshframed by PVC pipe. The transplantation frames stand on the ground at the horizontal level which paralleled to the ground at 1.5 meters above.

Chlorophyll fluorescence measurement: *In situ* chlorophyll *a* fluorescence of 10 thalli were measured all day-night for 31 hour, at 30 minute interval during the day light, and 1 hour at night. The measurement started from 04:00 hours and end at 11:00 hours during 6-7 June 2014 by using Mini-PAM (Walz, Germany). Zero value of quantum yield of PSII (ΦPSII) indicated that lichen was metabolic inactive.[123] ETR value indicates NP activity of lichen.[13]

Net Photosynthesis

photosynthesis rate of 5 lichen thalli were measured in laboratory under constant control condition of 350 μmol $m^{-2}s^{-1}$ of PAR, 90-100% thallus WC, and 25°C by using LI6400 (Licor inc., USA) according to Boonpeng et al. (2011).[5]

Growth measurement

Increasing thallus diameter as a measure of lichen growth was detected by photographic technic using Axioversion 4.1 LE during 6 May to 7 June 2014 according to Santanoo and Boonpragob (2013).[4]

Microclimate measurement

Light intensity was measured by Li-190SB (Licor Inc., air temperature and Relative Humidity by HMP 60 (Visala Inc.), and thallus temperatures by thermocouples type T (omega Inc.). The values were recorded by using CR10x Datalogger (Campbell Scientific, Inc., USA). The microclimatic condition was recorded every 5 minute during 6 to 7 June 2014 (Figure 1b.), concurrently along with measuring thallus water content and chlorophyll fluorescence.

Thallus water content was performed all day and night accompany with chlorophyll fluorescence measurement from 20 thalli of the horizontal aspect.

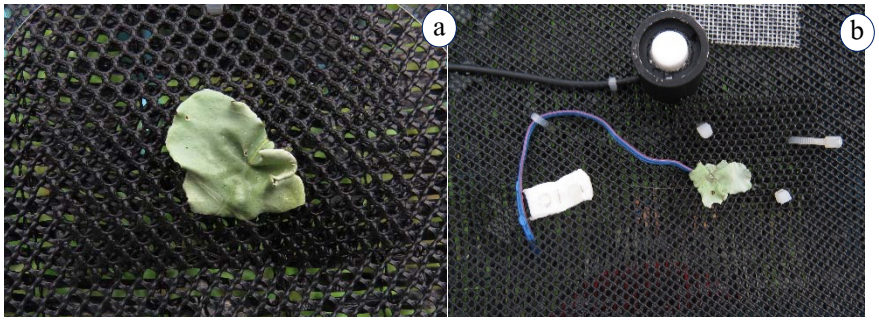

Figure 1. A small thallus of*P. tinctorum* transplanted on plastic net (a). Microclimate measurement, light and thallus temperature sensors were installed next to the lichen (b).

Results and Discussion: Previous study by Santanoo and Boonpragob (2013) reported that lichen *P. tinctorum* transplanted on man-made substrate laying horizontally and East has growth rate of 0.41 $mm/momth^{-1}$, which was higher than those of the North, South, and West aspects.[5] Transplantation under similar condition in this study the lichen had growth rate of 0.77 $mm/month^{-1}$. This study found that the lichen had higher growth rate than the previously reported.[5] It was probably caused by lichen thalli in this study were smaller, and the observation was taken in a shorter period, 3 months, at the end of summer and rainy season. This period most lichens have great growths due to rain water (Wannalux et al., 2013; Armstrong, 2006).[6,7]

Net photosynthesis of the transplanted lichen under the control optimum condition was 5.33 μmol CO_2 $m^{-2}s^{-1}$. Under same condition, Boonpeng et al. (2011) reported that NP of *P. tinctorum*was10 nmol CO_2 g_{dw}^{-1} s^{-1}. Recently, Boonpeng et al. (2014) reported that net photosynthesis of *P. tinctorum* varied year between 5.8 to 27.8 nmol CO_2 g_{dw}^{-1} s^{-1}, of which the highest value is measured in hot season.[8] In addition, Boonpeng et al. (2011) reported that light intensity above 50 μmol $m^{-2}s^{-1}$ and thallus WC higher than 10% of dry weight could activate photosynthesis of lichen achieving compensation point, of which carbon assimilate by photosynthesis equal to C lost by respiration. Therefore, these conditions were used to evaluate metabolic activities of lichen utilizing chlorophyll fluorescence parameters, ΦPSII and ETR. Daily metabolic activity could be divided into four periods (Figure 2 and Table 1) as follows:

1) *Photosynthetic active period*: ETR revealed that photosynthetic activity of lichen occurred in the morning during 06:00 to 11:00 hours, and lasted for ca. 5 hours. (Figure 2). The highest ETR occurred at 09:00 hours when light intensity was 229 μmol $m^{-2}s^{-1}$ and thallus WC was 57%. The optimum condition for maximum photosynthesis (P_{max}) of this lichen occurs at illumination 350 μmol $m^{-2}s^{-1}$, thallus WC 100% (Boonpeng et al., 2011). Under the ambient light and thallus WC, NP of the transplanted would be about 65%*, or 35% lower than P_{max}. Lang et al. (2001) reported that optimum condition for NP rarely occurred in nature.[1]

2) *Dry thallus respiration period*: low values of ΦPSII and WC of lichen close to zero, which indicated dry thallus respiration. It occurred during 11:00 to 16:00 hours and lasted for ca. 5 hours. The very low ETR was observed during 11:00 hours because of high light intensity, afterwards ETR was close to zero at 12:30hours, indicated that NP did not occurred. Lichen were dried out around midday under the ambient light 500 μmol $m^{-2}s^{-1}$ and high thallus temperature was 37°C. During dry thalli, Boonpeng et al. (2011) reported that respiration of this lichen occurred at very low rate between -0.17 and -0.32 nmol CO_2 g_{dw}^{-1} s^{-1}, which consumed about 1/31 fraction of carbon assimilated under P_{max}.

3) *Photosynthesis active period in late afternoon*: ΦPSII and WC slightly increased at relative humidity 75-80% during 16:00 to 18:00 hours, NP continued for 2 hours. It indicated that lichen was rehydrated and metabolic process became active again. The peak of ETR occurred again at 17:00 hours. When light intensity was 58 μmol $m^{-2}s^{-1}$ and WC was 8.3%. Light intensity was closed to compensation point, but WC is lower than compensation point. Therefore, assimilated carbon was probably used up by respiration. This assumption were referred to Lange (2003) found that in some time respiration occurred at low ETR.

4) *Dark respiration (DR) period*: high ΦPSII, close to maximum, occurred at night during 18:00 to 06:00 hours indicated that lichen was fully metabolic active, which lasted for 12 hours. During 18:00 to 22:00 hours, lichen absorb water from air humidity, which was 88 to 95% brought WC to reach about 10 to 25% dry wt. respectively. Dark respirationof this lichen occurred at 25% of WC, about -1 nmol CO_2 g_{dw}^{-1} s^{-1} was reported, which lower than 10 times of P_{max} (Boonpeng et al., 2011). When lichen were rewetted again by rain shower at 23:00hours and WC was increased to 244%. Thereafter, saturated thallus WC caused high ΦPSII and high rate of respiration. It consumed carbon as much as 1/2.5 portion of C assimilated by P_{max}.

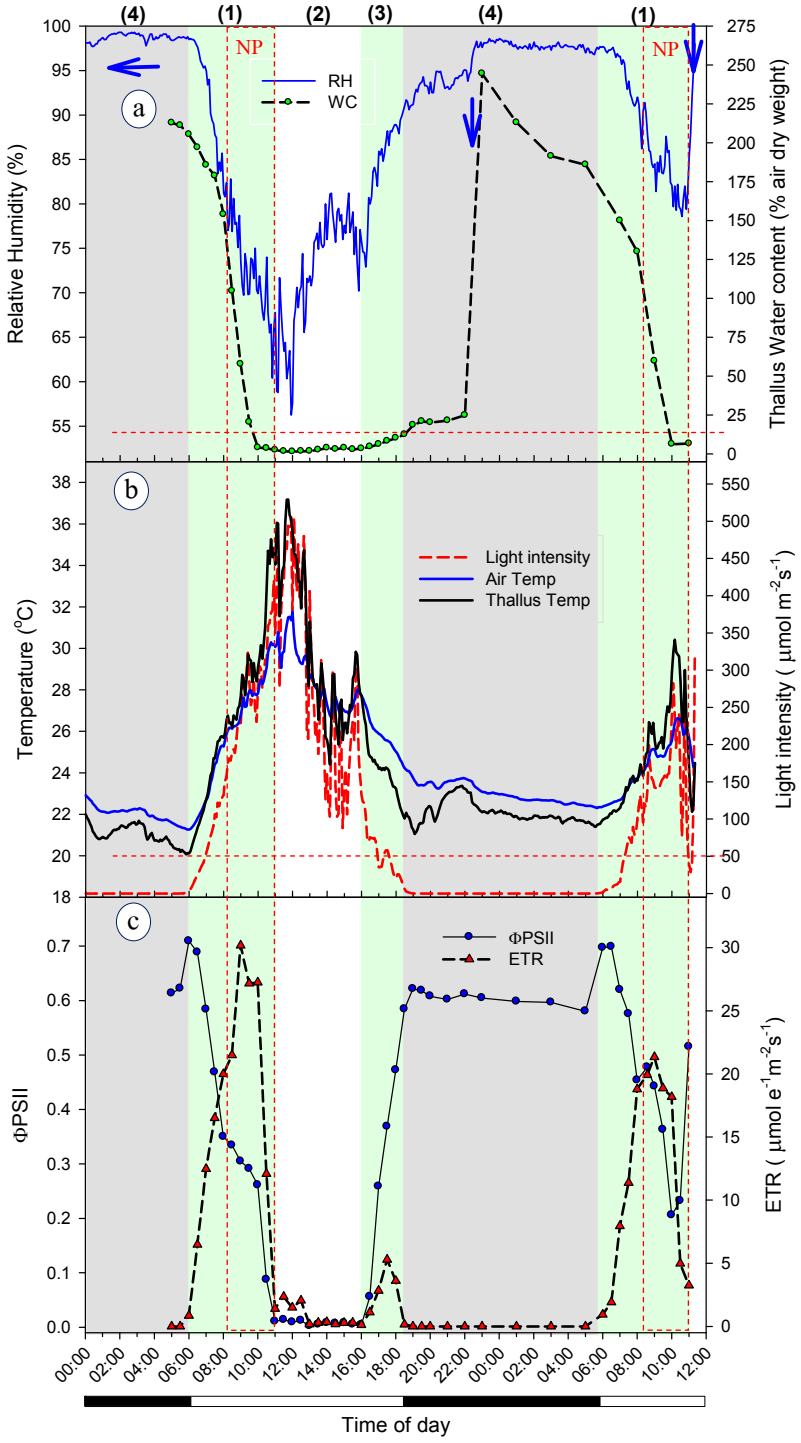

Figure 2. Microclimate in 6 to 7 June 2014; RH and WC (a), light intensity, air and thallus temperature (b). Chlorophyll fluorescence; ΦPSII and ETR (c). The blue arrows in figure A represented rain. The red dash line indicated light (>50 µmol $m^{-2}s^{-1}$) and WC (>10%) above compensation point. The red dash boxes is assessed positive NP concurrently. (1) is photosynthetic period in morning (6:00 to 10:00 hours), (2) is dry lichen period (11:00 to 16:00 hours), and (3) is photosynthetic period in afternoon (16:00 to 18:00 hours), (4) is respiration period (18:00 to 06:00 hours).

Table 1. Chlorophyll fluorescence parameter and microclimatic conditions during daily metabolic activity periods of transplanted lichen.

Parameter	Daily metabolic activity periods			
	Photosynthetic period in morning, 5 hr. (6:00 to 10:00 hours)	Dry lichen period, 5 hr. (10:00 to 16:00 hours)	Photosynthetic period in afternoon, 2 hr. (16:00 to 18:00 hours)	Respiration, 12 hr. (18:00 to 6:00 hours)
Chlorophyll fluorescence				
Average of ΦPSII	0.446	0.008	0.232	0.605
Average of ETR (μmol $e^{-1}m^{-2}s^{-1}$)	14.4	0.8	2.6	0.0
Microclimate				
Light intensity (μmol $m^{-2}s^{-1}$)	119	293	63	0
Thallus Temperature (^oC)	24.7	30.2	24.7	21.8
Air Temperature (^oC)	24.7	22.7	26.1	22.9
Relative Humidity (%)	87	76	83	97
Thallus water content (%)	104	2.5	6.5	107

Conclusion: this study underpinned the important of microclimate, especially, water and light during photosynthetic period of the transplanted lichen. Transplanted lichen had 7 hours for photosynthetic activity under illumination while, respiration period occurred longer than 2.4 times of photosynthetic period. However, respiration rate of lichen varied greatly due to thallus WC. Carbon lost by respiration was estimated to be a fraction of C assimilated by photosynthesis, ranged from 1/31 - 1/2.5, dry lichen was lower. Under ambient light and WC, net photosynthesis were about 65%* of P_{max}.Although, the optimum condition for P_{max} of *P. tinctorum* rarely occurred in the nature. This study revealed that the best condition for net photosynthesis of *P.tinctorum* of the transplanted habitat could be modify for achieve higher biomass production.

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