A_029_PF: WHICH IS THE SUITABLE TRANSPLANTED FRAME FOR CULTIVATING THE LICHEN *Parmotrema tinctorum* FOR SUSTAINABLE UTILIZATION?

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Abstract: Carbon assimilation in lichens depends largely on available atmospheric humidity. Maintaining optimal thallus water content can prolong carbon fixation and increase lichen production. The objective of this study was to develop transplanted frames that prolong optimal thallus water content. Three small and three large frames were made, standing 45° above the ground facing the East. Enclosing beneath each frame with either shading net, shading net lining with plastic sheet, or open (control). Six thalli of the lichen *Parmotrema tinctorum* were fixed over the surface of each frame. Thallus relative water content (RWC), chlorophyll fluorescence (Φ_{PSII}) and microclimate were measured from 5 a.m. to 1 p.m. for 4 days during 25 to 28 January 2019. The result showed that the frame that surrounded by the shading net lining with plastic sheet could maintain higher and longer humidity inside the frame. Consequently, the transplanted lichens on this frame showed evidently higher thallus water content than the others. Temperatures were not different among all frames. This result suggested that walling the lower surface of the frames with the combination of shading net and plastic sheet was suitable for lichen transplantation. This information is essential for cultivation of lichens for sustainable utilization. More importantly, further study under varying environment needs to be carefully assessed.

Introduction: Lichens are obligate symbiotic organisms between fungi and algae and/or cyanobacteria. They produce novel products that are different from other organisms and could be used for pharmaceutical purpose, cosmetic, foods, beverages etc.[1] In addition, lichens are well recognized as effective bioindicators of air quality.[2]

The lichen *Parmotrema tinctorum* (Despr. ex Nyl.) Hale used as an experimental material for this study distributes across all ecosystems in Thailand and was commonly found in Khao Yai National Park. It has been utilized successfully for silk dying and a bioindicator of air quality.[2] It also metabolizes secondary substances that have potential to be used for pharmaceutics. However, the growth rate of the lichen is very slow (9.86-10.71 mm/yr.)[3] because it has a relatively short period of time for carbon assimilation. Photosynthesis of lichens depends largely on atmospheric moisture which terminates in the late morning when air humidity drops and thallus runs out of water.[4,5] As such, natural lichen biomass is not sufficient for utilization. It is essential to develop method that enable to increase lichen production such as prolong optimal thallus water content for longer and higher carbon assimilation. Previous studies revealed that soil watering under transplanted frames could increase lichen growth rate,[6] nevertheless the growing biomass remained under utilization demand, as a result further experiments are needed.

The main objective of this study was to develop transplanted frames that allow longer optimal thallus water content that essential for longer photosynthesis process.

Methodology: This experiment was performed at the secondary forest in Khao Yai National Park (KYPN), Thailand.

Six transplanted frames made up from PVC pipes, lining the square surface area with black polyethylene net and a 50% black shading net. They composed of two sizes, three large (L) frames (200x70 cm or 1.4 m^2) and three small (S) frames (50x70 cm or 0.35 m^2) (Figure 1a and Table 1). Each frame stands 45° inclination on the ground, facing east. All four sides underneath the frames were enclosed by 50% black shading net and/or a plastic sheet. Six

thalli of *P. tinctorum* were fixed on top of each frame for measuring thallus water content and chlorophyll fluorescence. All frames stood under 50% black shading net.

Table 1. Condition of the transplanted frames.

Frame No.	Size	Underneath frame walling	abbreviation
1	Small (50x70 cm)	None (Control)	S_C
2	Small (50x70 cm)	50% black shading net	S_N
3	Small (50x70 cm)	Plastic sheet and 50% black shading net	S_{P+N}
4	Large (200x70 cm)	None (Control)	L_C
5	Large (200x70 cm)	50% black shading net	L_N
6	Large (200x70 cm)	Plastic sheet and 50% black shading net	L_{P+N}

Approximately 10 liters of natural water was sprinkled with flow rate of 1 L/min. beneath each small frame. Whilst the larger frames received a double supplied of 20 liters. The watering was applied during 7 and 9 a.m. every day during experiment. Thallus water content was calculated from the six lichen thalli fixing on each frame using the formula according to Barták,[6] They were expressed as a relative water content (RWC); (FWa-DW)/(FWw-DW) x 100 (% water status of thalli), where FWa is fresh weight of actually hydrated thallus, FWw is fresh weight of fully hydrated thallus of the day, and DW is dry weight of dehydrated thallus. Chlorophyll fluorescence parameter (yield or Φ_{PSII}) that was used to determine the physiological active period of the lichens were conducted by a Pulse Amplitude Modulation Fluorometer, MINI-PAM (Walz Inc. Germany). Relative humidity (RH), air temperature (temp.) inside and outside the transplanted frames were recorded, while photosynthetically active radiation (PAR) was measured over the frame. This experiment was repeated for 4 days during 25 to 28 January 2019, and from 5 a.m. to 1 p.m.

Statistical differences of the data were tested using Student's t-test by IBM SPSS Statistics version 20 (IBM Corporation, USA), and graphs were created by SigmaPlot version 11 (Systat Software, Inc., USA).

Figure 1. Lichen transplanting
(a) Thallus of the lichen *Parmotrema tinctorum*. (b) fragmented thalli of lichen over the large and small transplanted frames, and (c) measuring microclimate.

Results and Discussion: The consequences of frame size on relative humidity (RH) underneath the surface, and relative water content (RWC) of the lichens transplanted over it remain unclear. The RH and RWC were similar when underneath the frames were open (Figure 2). However, with only enclosing net below the frame caused RH and RWC of the large frame was higher than the small one. Contradictory, this effect was reversed in the frames with surrounded by net and plastic underneath.

It was clear that enclosing underneath the frames with a combination of net and plastic sheet (P+N) resulted in higher humidity under the frames and the lichen thalli than those without them (Figure 2 and Table 2). Thallus water content over the small frame that the underneath bound with net and plastic sheet (S_{P+N}) had RWC significantly higher ($p<0.001$) than those over the large frame with similar bounding (L_{P+N}). In addition, Table 2 shows that the S_{P+N} could maintain inside RH above 80% for about 45 minutes longer than the control (S_C). Similarly, at 9 a.m., the transplanted lichens had evidently higher RWC (46%) than the others. This amount of water was enough to maintain its high physiological activity, as showed by the chlorophyll fluorescence parameter (Φ_{PSII}), for almost double at 9 a.m. This was probably because of; (i) the smaller surface area of the transplanted frame delayed water evaporation; (ii) enclosing underneath the frame with plastic sheet and shading net helped to hold high moisture. It indicated that the S_{P+N} frame could prolong optimal thallus water content, and consequently extend period of carbon assimilation of the lichen.[4]

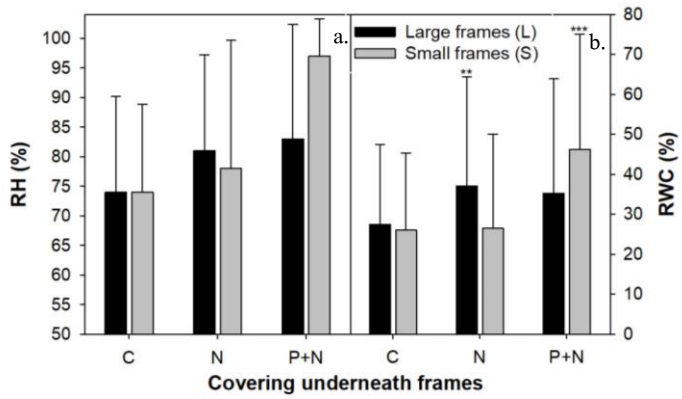

Figure 2. Microclimate and thallus water content of the lichen at the transplanted frames (a) Relative humidity (RH) inside the frames, and (b) relative water content (RWC, % water status of thalli) of the lichen thalli at 9 a.m. Denotes type of wall covering under the frames: C control, open without covering, N enclosed with 50% black shading net, and P+N bound with plastic sheet and 50% black shading net. Asterisks on top of the bars denote statistically significant differences between the pair (** $p<0.01$, *** $p<0.001$) by Student's t-test.

Table 2. Time and period of relative humidity (RH) below 80% under the transplanted frame, thallus water content (RWC) and chlorophyll fluorescence (Φ_{PSII}) of *Parmotrema tinctorum* at 7 and 9 a.m.

Transplanted frame	Time RH below 80% (a.m.)	80% RH longer than Control (min.)	RWC (%)		Φ_{PSII}	
			7 a.m.	9 a.m.	7 a.m.	9 a.m.
L_C (Control)	8.51	-	99	27	0.71	0.28
L_N	9.01	10	97	37	0.73	0.27
L_{P+N}	9.10	18	99	35	0.69	0.26
S_C (Control)	8.52	-	99	26	0.72	0.23
S_N	8.57	5	98	27	0.73	0.32
S_{P+N}	9.37	45	96	46	0.70	0.48

Previous studies observed that frames walled with plastic sheet accumulated heat beneath them. They enhanced the respiration rates. This study modified the former ones by

using 50% black shading net lining with the plastic sheet. Assuming that the shading net may reduce light intensity and temperature. We found that temperature inside the current frames were comparable with the open frames (control), all of which were 1-2 °C above the ambient air (Figure 3b).

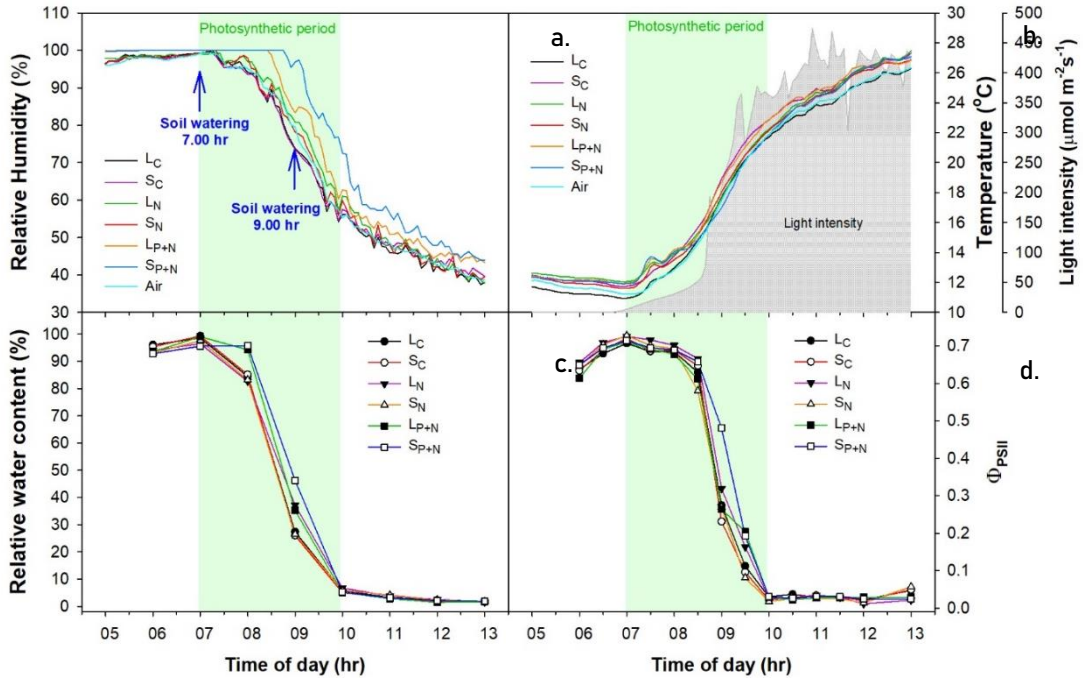

Figure 3. Microclimate and efficiency of photosystem of the lichen at the transplanted frames (a) Relative humidity (RH) (b) temperature and light intensity (c) thallus relative water content (RWC) and (d) efficiency of PSII (Φ_{PSII}) of the lichens. Data was measured and recorded at 5 a.m. to 13 a.m. during 25 to 28 January 2019. Denotes type of wall covering under the large (L) and small (S) frames: C control, open without covering, N enclosed with 50% black shading net, and P+N bound with plastic sheet and 50% black shading net.

Watering the ground under the transplanted frames could increase growth rates of the transplanted lichens.[3] However, maximizing growth rate of the transplanted lichen by an extra supply of water remained controversial. Soil watering at 7 a.m. did not increase RH (Figure 3a) because air humidity still saturated (98-100% of RH). Providing water at 9 a.m. increased ca. 5-6% of air humidity in the L_{P+N} and S_{P+N} for a short period time. However, it could not help to retain thallus water content. This was probably caused by water was used up readily for photosynthesis in the early morning under the sunlight and unable to quickly reabsorb from the frame or the atmosphere, including relatively low volume (10 liters/frame) of soil watering. Therefore, we suggest providing soil water at 7-8 a.m., or intermittently until 9 a.m. because the thallus remains hydrated and physiologically active. Delayed watering, when thallus almost dried out and inactive, make it difficult to reactivate again other than extra respiration cost.

The lichens from all frames dried out at about 10 a.m. Their photosynthesis terminated as showed by the chlorophyll fluorescence parameter (Figure 3d). This was because of low atmospheric humidity resulted from intense sunlight and elevated temperature,[4,5] and more importantly, used up of water by photosynthesis.

Conclusions: This experiment suggested that time and volume of soil watering, 7 a.m. and 10 liters, under the transplanted frames of 50x70 cm. enclosed by plastic and shading net under the surface was the best for cultivating lichens. However, watering pattern and frame size could be further modified under different environment.

References:
1. Rankovic´ B, Studzińska-Sroka E, FaŁtynowicz W. 2015;16:34-43.
2. Boonpeng C, Polyiam W, Sriviboon C, Sangiamdee D, Watthana S, Nimis PL, Boonpragob K. 2017;24:12393–12404.
3. Phaengphech M, Polyiam W, Boonpargob K. 2018;44:226-230.
4. Palmqvist K, Sundberg B. 2000;23:1–14.
5. Phaengphech M, Fuangkaew P, Polyiam W, Watthana S, Boonpargob K. 2016;42: 611-615.
6. Barták M. 2014;39:379-400.

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