การย้ายปลูกไลเคน *Parmotrema tinctorum* บนตาข่ายโปร่ง ณ อุทยานแห่งชาติเขาใหญ่

Transplantation of lichen *Parmotrema tinctorum* on ventilated nets at Khao Yai National Park

มงคล แผงเพ็ชร ศรัทธารา หัตถีรัตน์ และกัณฑรีย์ บุญประกอบ
Mongkol Pangpet Sattara Hattirat and Kansri Boonpragob
Department of Biology, Faculty of Science, Ramkhamhaeng University, Bangkapi, Bangkok 10240, Thailand; Fax: (662)310-8395, e-mail address: mongkolpp@gmail.com

บทคัดย่อ: ไลเคน *Parmotrema tinctorum* มีศักยภาพนำไปใช้ประโยชน์ได้มาก พบแพร่กระจายทุกระบบนิเวศในอุทยานแห่งชาติเขาใหญ่ และมีอัตราการเติบโตสูงกว่าไลเคนชนิดอื่น การศึกษานี้มีวัตถุประสงค์เพื่อทดสอบการเพิ่มผลผลิตของไลเคนชนิดนี้ ด้วยการย้ายปลูกบนตาข่ายพลาสติกที่มีการระบายอากาศดี เพื่อลดการแก่งแย่งที่อยู่อาศัยของสาหร่ายที่เติบโตได้เร็วกว่าเมื่อย้ายปลูกบนวัสดุซึ่งเก็บความชื้นได้ดี การย้ายปลูกเริ่มทำในเดือน กรกฎาคม 2548 โดยใช้ส่วนปลายแทลลัสจำนวน 114 ชิ้น บันทึกการเหลือรอดและอัตราการเติบโตจากภาพถ่ายในเดือนที่ 6 15 21 29 และ 36 หลังการย้ายปลูกโดยสิ้นสุดเมื่อกรกฎาคม 2551 พบว่าไลเคนเหลือรอดจำนวน 87 42 27 19 และ 17 แทลลัสตามลำดับ ความเข้มแสงและความชื้นสัมพัทธ์ในอากาศส่งผลให้ไลเคนเกิดกระบวนการสังเคราะห์ด้วยแสงประมาณ 3-4 ชั่วโมงในช่วงเช้า อัตราการเติบโตเฉลี่ยของแทลลัสที่เหลือรอดคือ 0.42 มิลลิเมตรต่อเดือน (มม./เดือน) อัตราการเติบโตสูงสุด 1.47 มม./เดือน อัตราการเติบโตเฉลี่ยสูงหลังช่วงฤดูฝน 0.55 มม./เดือน และต่ำช่วงหลังฤดูแล้ง 0.35 มม/เดือน การตายเกิดขึ้นสูงหลังย้ายปลูก 15 เดือน โดยเฉพาะในแทลลัสขนาดเล็ก เนื่องจากมีการสูญเสียน้ำเร็วกว่าแทลลัสขนาดใหญ่ การย้ายปลูกไลเคนเพื่อเพิ่มผลผลิตบนตาข่ายโปร่งจึงต้องคำนึงถึงขนาดของแทลลัส ขนาดของช่องตาข่าย ร่วมกับปัจจัยทางสภาพแวดล้อมอื่น เช่น ความเข้มแสง ระยะเวลาการได้รับแสง และระยะเวลาที่แทลลัสเกิดการสูญเสียน้ำ

Abstract: The lichen *Parmotrema tinctorum* has high prospect for utilizations of its novel products. It is commonly distributed in every forest types in Khao Yai National Park and has high growth rates. This study aimed to increase biomass production of this lichen by transplanting thallus fragments on plastic nets which were artificial substrate that provided good ventilation. This condition minimized spatial competition of an algae found in previous study to, colonize moist substrates faster than lichens. The transplant started in a secondary forest (SF) in July 2005 by using the 114 pieces of active thallus margins attached to plastic nets. Subsequently, thalus expansion was recorded by photography after 6, 15, 29 and 36 months after transplantation, which terminated in July 2008. It was found that 87, 42, 20 and 17 thalli survived respectively during these periods. Microclimate at the transplantation site (87% RH, 23 ^{o}C and 140 μmol $m^{-2}s^{-1}$) indicated that lichen photosynthetic activity probably occurred for 3-4 hours in the morning. The average growth rate of surviving thalli was 0.42 mm/month. The highest growth rate was 1.47 mm/month. The average growth rates were high after the wet season (0.55 mm/month), and low after the dry season (0.35 mm/month). Deaths occurred extensively within 15 months of transplantation, particularly in small thalli. This was probably due to low resistance to rapid evaporation of small thalli when placed on a large-pored

substrate. The study suggests that thallus sizes and pore sizes of a net substrate, which influence evaporation rates, in accordance with other factors, e.g. light intensity and illumination time, should be taken into account for successful transplantation.

Introduction: Despite their bioactive products in medicinal and agricultural aspects, lichens grow slowly and provide insufficient biomass for sustainable utilization. Attempts to transplant them to increase biomass production had been made [1]. However, the factors that govern successful transplantation and production remained unclear. Also, the knowledge of optimum conditions for tropical species were scarce. An observation on transplantation of thallus fragments of the lichen *Parmotrema tinctorum* on tree barks in secondary forests (SF) of Khao Yai National Park (KYPN) showed that they had high survival percentages, comparing to those transplanted in other forest types [1]. The natural average maximum growth rate of *P. tinctorum* in SF (KYPN) was also the highest [2]. SF was considered as having a microclimate that favored growth of *P. tinctorum* and was then used as a transplantation site in this study. There had been an attempt to transplant *P. tinctorum* on artificial substrates, e.g. ceramic tiles, plastic plates and plastic nets [3]. Thalli on ceramic tiles had low survival because they could not compete with an alga *Scytonema* sp., which established rapidly on the moist substrates. Transplanted thalli on plastic plates were hardly successful probably due to excessive water accumulating on unventilated substrates, blocking gas exchange of the thalli. There was also little success with the thalli transplanted on plastic plates because the substrates provided less ventilation and kept water underneath the thalli. This resulted in high respiration but little photosynthesis, causing death of lichen thalli. Thalli transplanted on plastic nets had the highest growth, probably due to adequate ventilation. The present study, therefore, was planned to investigate the effects of plastic net on survival and growth pattern of transplanted thalli of *P. tinctorum* in SF. The hypothesis was that thallus fragments of lichens could be transplanted and grow successfully on artificial substrate with good ventilation.

Methodology: Apical lobes of about 1.7 cm in diameters of *Parmotrema tinctorum* (Despr. ex Nyl.) Hale collected from secondary forest at Khao Yai National Park (KYNP) were attached on 114 pieces of 10x10 cm^2 pieces of plastic nets. Nylon threads were used for attachment. The net substrates have pores 4x3 mm^2 in size intermittent with threads 1 mm in diameter. They were attached horizontally on two parallel wires 1.5 m above the ground at the same forest in July 2005. Green shading nets were placed at about 0.5 m above the transplanting nets. Growth was measured from digital photographs taken with Sony P73, lens 6-18 mm and AxioVision LE Rel. 4.1 software. The data discussed here were from 27 lichen thalli that survived after 20 months of transplantation. Microclimate (illumination, temperature and moisture) at transplantation sites were measured using Licor-1400. Chlorophyll fluorescence, which was used to indicate photosynthetic active period, which Electron quenching in photosynthetic activity was determined using MINI-PAM (Walz, Effeltrich, Germany).

Results, Discussion and Conclusion:

Survival, growth rates and chlorophyll fluorescence

Of the 114 initial transplanted lichen thalli lichen lobes transplanted on the plastic net, only 27, accounting for 24%, lobes survived and continued to grow after 21 months of transplantation. Death of transplanted thalli occurred extensively within six and fifteen months with 23% and 63% recorded respectively.

The surviving thalli had high average growth rates of 0.55 mm/month during a period of rainy season, and low average growth rates of 0.35 mm/month during a dry season (Fig 1). A similar seasonal growth pattern is observed in the natural habitats [2].

Growth rates tend to increased with increasing thallus sizes (Table 1), indicating that they approach rapid growth phase, a short but critical time in lichen life span when they must occupy their habitat quickly [3]. The highest growth rate was from the third year of transplantation. The timing is similar to that of natural lichens [4].

Growth occurred markedly at the former apical region of the transplanted lobes (Fig. 1). There was no thallus development observed at the cut areas (Table 1). This was different from Kon *et al* (2005) who reported growth at edges of the transplanted thalli of *P. tinctorum* which were cut into square fragments. Their transplanted lichen fragments grew from all of the four cut rims.

The diameters of the surviving thallus fragments increased continuously in the first 29 months then decreased thereafter (Table 1). Their average and maximum growth rates were 0.42 and 1.47 mm/month, respectively. These were less than the rates observed from lichens in natural habitats (1.5 and 2.6 mm/month) [2]. Therefore, the growth pattern of the surviving thalli was consistent to the naturally growing ones, having more growth in wet seasons, but their overall productivity decreased. This is because the transplanted thalli required an establishment period on a new substrate, resulting in retarded growth.

Table 1. Sizes and growth rates of *P. tinctorum* (27 surviving thalli) during 35 months after transplantation (July 2005 to July 2008)

Months of observation (mos./year)	Total numbers of thalli	Thallus sizes (average)		Growth rates (mm/month)	
		Area (cm^2)	Diameter (cm)	Average	maximum
1 (8/2005)	27	2.32	1.73	-	-
6 (1/2006)	27	2.83	1.90	0.39	0.8
15 (10/2006)	27	4.52	2.39	0.56	0.94
21 (4/2007)	27	5.15	2.54	0.27	1
29 (12/2007)	19	6.77	2.90	0.54	1.43
36 (7/2008)	17	6.57	2.75	0.39	1.47
Mean				0.42	1.23

Cause of death

The high death percentages could likely be due to the sizes of transplanted lobes. According to studies of lichen growth made by Osathanon (2002) and Wetchasart (unpublished, 2006) [3] revealed that *P. tinctorum* with thallus diameters less than 3 cm showed signs of death and began to disintegrate in 2 years after growth's monitoring started. having been recorded for growth. In 6 years, all of the smaller thalli completely died. Contrastingly, death of larger thalli (diameter > 3 cm) were recorded after 5 years of observation. After 6 years, overall death of large *P. tinctorum* thalli accounted for only 25%. Some of the larger thalli continue growing through their ninth years.

A number of studies suggested that small thalli had higher rates of water loss. This was due to their low boundary layer resistance [5] and thin cortex and medulla [6]. As the transplanted thalli in the present study were small with diameters of only about 2 cm, water loss could have been the cause of high proportion of death thalli due to the nature of plastic net substrate which dry out quickly.

Although maintaining sufficient moisture in thalli was crucial photosynthetic activities of lichen, transplantation on substrates which hold excess moisture resulted in higher proportion of death [2]. *P. tinctorum* transplanted on polycarbonate plates were likely to be oversaturated as the plates provided less ventilation. Excessive water was trapped on the plates, particularly during rainy season. This blocked CO_2 diffusion to carboxylation sites of algae. In addition, *P. tinctorum*, which depended on rhizenes for substrate attachment, detached easily from the smooth surface of polycarbonate plates. Ceramic tiles, with high porosity, could hold more water and provided moderate ventilation. However, the basic nature of ceramic tiles favored growth of a fast growing alga (*Scytonema* sp.), which succeeded in spatial competition over the slow growing lichens.

Microclimatic conditions (Fig 2.) and chlorophyll fluorescence analysis suggested that photosynthetic activity of transplanted thalli lasted 3-4 hours during the morning period when relative humidity was more than 70% [7]. The duration was shorter than that observed in *P. tinctorum* growing on barks and rocks under similar level of light intensity in SF. Their photosynthesis also terminated when RH was less than 70%, but such condition normally occurred later during the day at around noon [6]. This was because barks and rocks could supply prolonged moist condition for the lichens.

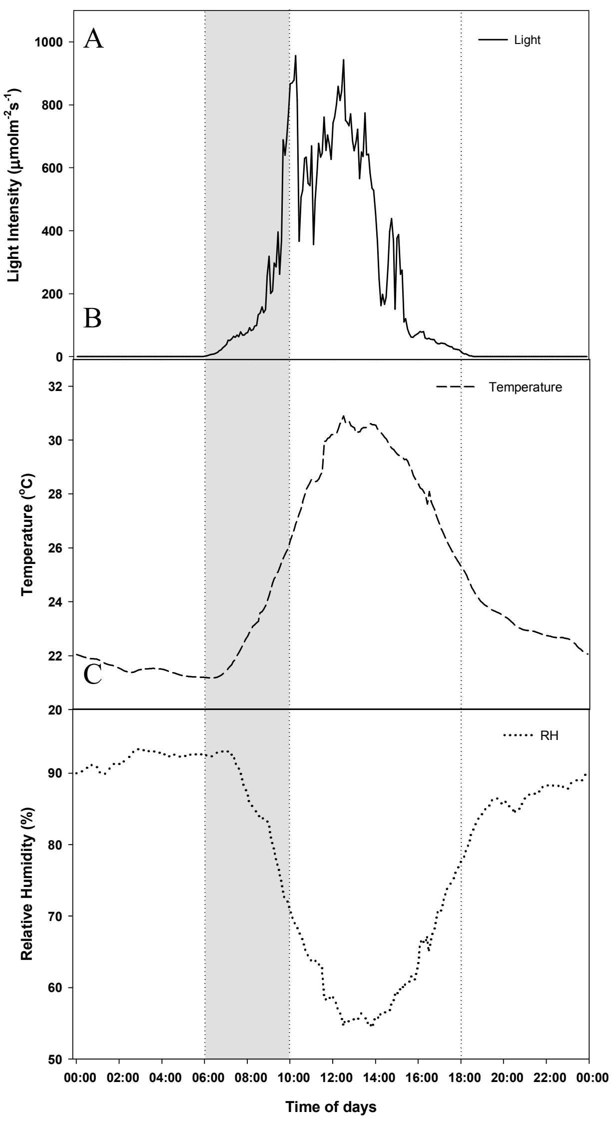

Figure2. Microclimate at transplantation sites, averaging from the data measured on 26-28 April 2006 and 19-20 July 2008; (A) light intensity, (B) temperature and (C) relative humidity. The grey area corresponds to photosynthetic active period as indicate by chlorophyll fluorescence measurements (6-10 am).

In conclusion, this study suggested that transplantation of *P. tinctorum* on ventilated substrates was possible. Nevertheless, thallus size and water loss during photosynthetic periods were essential factors for successful transplantation and needed careful consideration. Plastic nets with smaller pore sizes, which could reduce evaporation from thallus, should be an appropriate choice for future transplantation.

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Keywords: Chlorophyll fluorescence, microclimate, photosynthetic period, lichen growth rate, growth pattern, apical growth, thallus size, water loss, lichen transplantation, artificial substrates

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