CHAPTER 3

METHODOLOGY

Study Sites

Khao Yai National Park (KYNP) is the first of national park of Thailand, covering an area 2,165 km^2 and encompassing 11 districts belonging to four provinces; Nakhon Ratchasima, Saraburi, Nakhon Nayok, and Prachinburi. The location is between 14° 05′ and 14° 35′ N latitude, 101° 05′ and 101° 50′ E longitude, at elevation about 200 to 1,351 m.a.s.l. (see Figure 3). KYNP consists of various ecosystem such as; Tropical Rain Forest (TRF), Dry Evergreen Forest (DEF), Lower Mountane Forest (LMF), Secondary Forest (SF), Dry Dipterocarp Forest (DDF), Mixed Deciduous Forest (MDF) and Plantation (P) as shown in Figure 4.

Kongkanda Chayamarit (2006, p. 11) described climate of Khao Yai National Park as follow;

> The Rainy Season is caused by the Southwest monsoon which brings heavy rainfalls from May to October. During this period it rains almost every day; the atmosphere is very humid and temperatures are high (average daytime temperature of ca. 27 °C). After daily rains, air is clean and visibility is excellent. More than three quarters of annual precipitation falls during monsoon. The Park Headquarters, for example, records more than 84% of annual rainfall for the period of

May to October. The Cold Season (November to February), is characterized by clear, sunny and cool weather. Rain showers only occur sporadically; in the driest months (December and January), rainfall averages no more than 15 mm per month. Average daytime temperatures are around 22 °C, but in the coolest months of December and January temperatures may not exceed ca. 17 °C during the day and drop much lower during the night, sometimes to less than 10 °C. The Hot Season (March to April), is characterized by hot, dry and often windy. Average temperatures in these two hottest months are around 28 °C; absolute temperatures exceeding 30 °C are not uncommon on certain days.

However, the average air temperature of 25.4°C, while mean relative humidity of 91% are reported. (Wisut Suwannapinunt & Somkid Siripatanadilok, 1982). Besides, Kongkanda Chayamarit (as cited in Gray et al., 1994) described that annual rainfall within the Park varies greatly. At the Park center, for example, mean annual rainfall was 2,270 mm., however, rain in other areas of the Park may be averaged around 3,000 mm a year. Moreover, they suggested that it might be over 4,000 mm on the highest ridges (as cited in Smitinand, 1968).

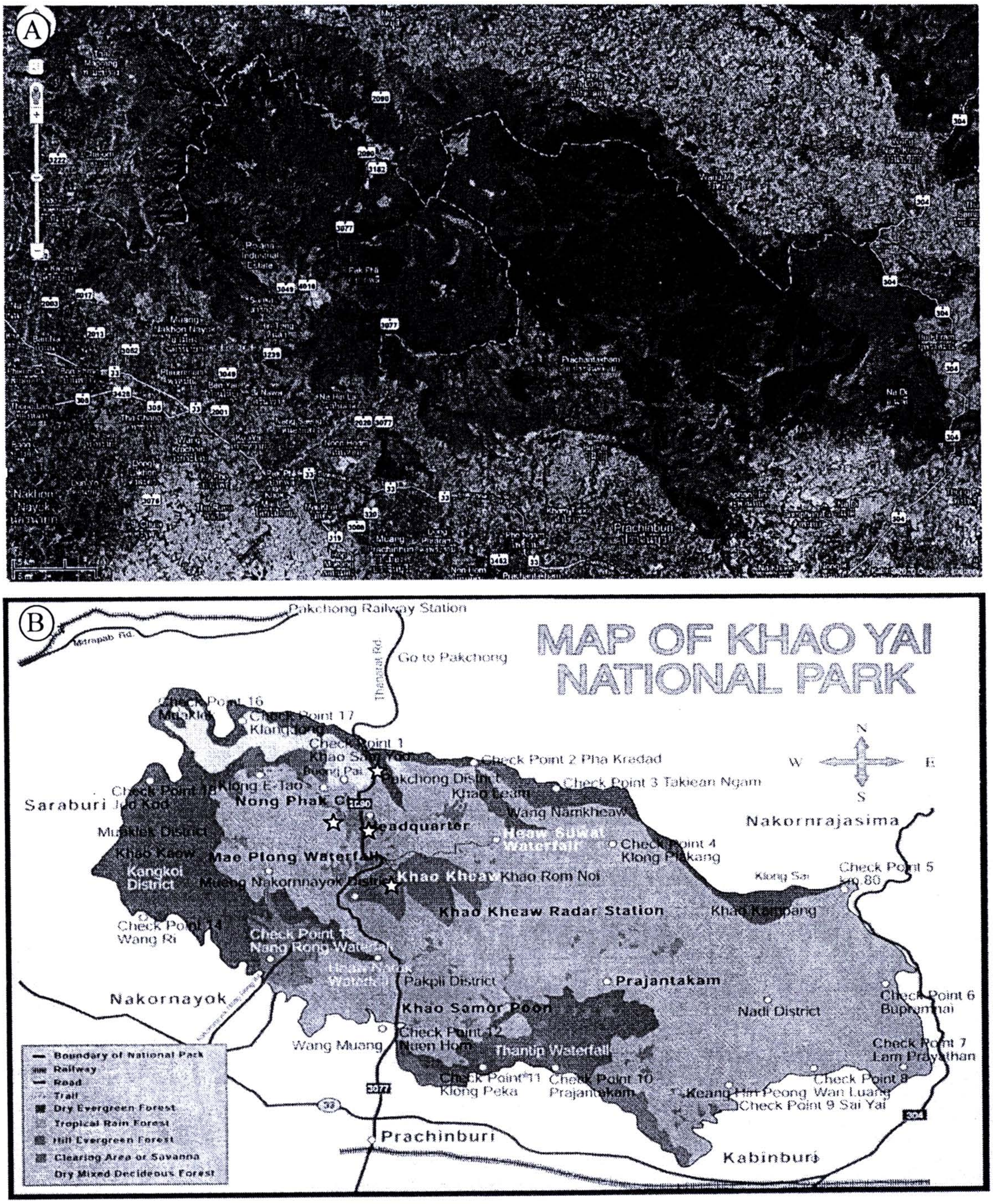

Figure 3 Map of Khao Yai National Park.

Note. From Google Earth 5.2.1.1588; aerial photograph (A).

Map of Khao Yai National Park. Retrieved November 23, 2009, from http://www.chalethill.com/images/sightseeing/map_khaoyai_eng.gif. Different forest types of forests (B). Stars located the four study sites.

Forest Types of Khao Yai National Park

Tropical Rain Forest

Tropical rain forest has been identified by physiognomic characteristics and indicator species of plants (see Figure 4). This forest is composed of evergreen plant species. It needs rainfall more than 1,600 mm per year, with continuously fall over 8 months of rainy season. The canopies layers exceed 30 meters in height (Dokrak Marod & Utits Kutintara, 2009, pp. 434-435). Kongkanda Chayamarit (as cited on Thawatchai Santisuk, 1988) described character of the Tropical rain forest at Khao Yai National Park. This type of forest is widespread, covering most areas of the park at altitude between 400 to 1,000 m.a.s.l. The flora of this forest is composed of several Dipterocarpaceae, including species of *Dipterocarpus*, dominating on emergent layer of this forest. Other trees that are frequently seen including *Alstonia scholaris* (Apocynaceae), *Carallia brachiata* (Rhizophoraceae) and *Ficus* species (Moraceae). In addition, some deciduous species can be found such as *Tetrameles nudiflora* (Tetramelaceae), *Pterocymbium javanicum* (Sterculiaceae) or *Acrocarpus fraxinifolius* (Leguminosae Caesalpinioideae). The undergrowth species is dense and rich in herbs and ferns. Acanthaceae are common, as well as several monocots of Alpinia and Zingiberaceae.

Figure 4 Tropical rain forest (Warren trail) at Khao Yai National Park.

Dry Evergreen Forest

Dry Evergreen Forest is identified by structure of plant communities by (Dokrak Marod & Utits Kutintara, 2009, p. 446). This forest composes of deciduous and evergreen trees (see Figure 5). The deciduous plants shed their leaves during exposure to dry period of approximately 3-4 months. The average rainfall is 1,000 to 2,000 mm per year (Nimitr Osathanon, 2002). Kongkanda Chayamarit (2006) described the Dry evergreen forest at Khao Yai National Park as follow: It is found at approximately 400 to 600 m.a.s.l. altitudes. Plants species of this forest composes of *Afzelia xylocarpa* (Leguminosae-Caesalpinioideae), *Pterocarpus macrocarpus* Leguminosae-Papilionoideae), *Melia azedarach* (Meliaceae), *Lagerstroemia calyculata* (Lythraceae), *Holarrhena pubescens* (Apocynaceae) or *Gmelina arborea* (Labiatae). In other areas, numerous Dipterocarpaceae can be found including

Dipterocarpus, Vatica and *Shorea* species, *Parkia* (Leguminosae-Mimosoideae), *Aglaia* (Meliaceae) and *Erythrophleum succirubrum* (Leguminosae-Papilionoideae). Several palms *(Areca triandra, Corypha,* etc.) occur in this forest type, and the ground flora is rich in various monocots, especially Zingiberaceae.

Figure 5 Dry Evergreen Forest (adjoining Km 33) at Khao Yai National Park.

Lower Montane Forest

This forest type associated with a cold climate (Dokrak Marod & Utits Kutintara, 2009, pp. 456-457). The Lower Montane Forest of Khao Yai National Park is located at Khao Keaw. Average temperature is 20°C and some areas are frequently covered with clouds (see Figure 6). Rainfall and humidity are high at this altitude, as a result tree trunks are covered by ferns, mosses, lichens and epiphytes (Kamol Suwanpatra, 2006).

Kongkanda Chayamarit (2006, p. 19) described this forest as following:

> Lower Montane Forest is rather dense, but the trees are smaller and often tend to be stunted. The Dipterocarpaceae is no longer present at these high altitudes and are replaced by various gymnosperms (*Podocarpus* s.l., *Dacrydium,* all Podocarpaceae). Many of the Fagaceae noted at higher elevations in the Moist Seasonal Rain Forest also extend to the highest areas. *Annesla fragrans* (Theaceae) is one of the dominant tall trees. Other common trees include *Olea brachiata* (Oleaceae), *Ficus altissima* (Moraceae) and the endemic *Reevesia pubescens* var. *siamensis* (Sterculiaceae). The understory is dense, consisting of many small trees and shrubs, amongst them are *Litsea* (Lauraceae), *Syzygium* and *Rhodomyrtus tomentosa* (both Myrtaceae), *Vaccinium* (Ericaceae), *Psychotria* (Rubiaceae) and *Pittosporum* (Pittosporaceae).

Figure 6 Lower Montane Forest (Khao Keaw) at Khao Yai National Park.

Secondary Forest

The Secondary Forest at Khao Yai National Park occurs after clear cutting of tropical rain forests (see Figure 7). Moore (2008) suggested that after old growth forest (tropical rain forest) were destroyed, the canopies are opened, enhancing the fast-growing trees to occupy the area. The area could be covered by small trees for many decades. Temperature and relative humidity of this forest has never been recorded. Kongkanda Chayamarit (2006) described that the Secondary Forest at Khao Yai National Park as follow: The Secondary Forest was succeed by pioneer plants , including the Euphorbiaceae genera Macaranga and Mallotus, *Duabanga grandiflora*, *Anthocephalus chinensis* and *Talipariti macrophyllus*.

Figure 7 Secondary Forest (Nong King) at Khao Yai National Park.

Lichen Materials and Transplantation Methods

This study use vegetative propagules of lichens consisting of thallus fragments and diasporas, of which the latter composed of isidia and soredia. These lichen propagules were prepared for transplantation in various sites according to the transplantation design that was described in latter section.

Thallus Fragments

Growing zone at the margin of lichen thalli were used. Lobe tips approximately 1-4 cm^2 (depending on lobe sizes), cleaned by bush, were used for propagation. The transplant materials were fixed on bark of tree trunks by nylon monofilament (see Figure 8).

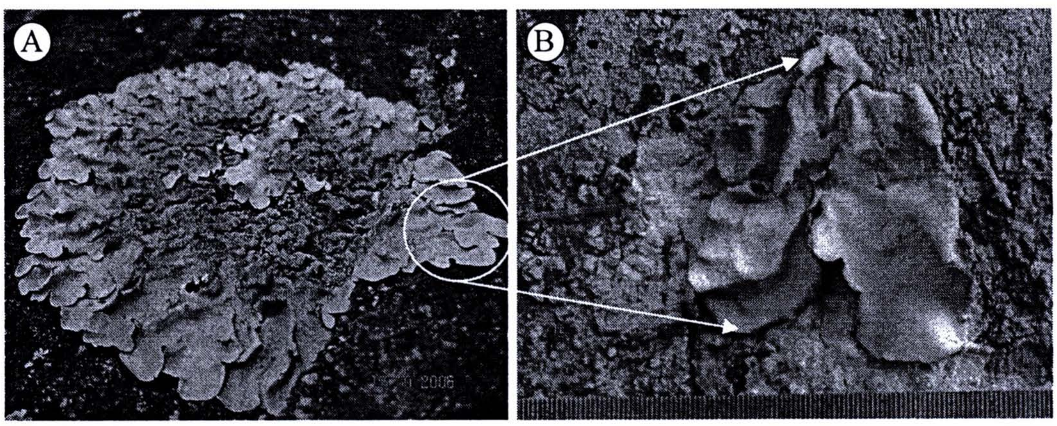

Figure 8 Lichens thallus (A) and thallus fragment (B) of *P. tinctorum* used for transplantation.

Isidia and Soredia

Isidia of *Parmotrema tinctorum* (see Figure 8A) and *P. sulphuratum* and soredia of *P. praesorediosum* and *P. sancti-angelii* (see Figure 9A-C) were used for transplantation. Isidia and soredia were sown on Double-Sided Glue Tape (DSGT) of 2x2.5 cm^2 (see Figure 10) and fixed on selected substrates. The materials were collected from SF during November to December 2003.

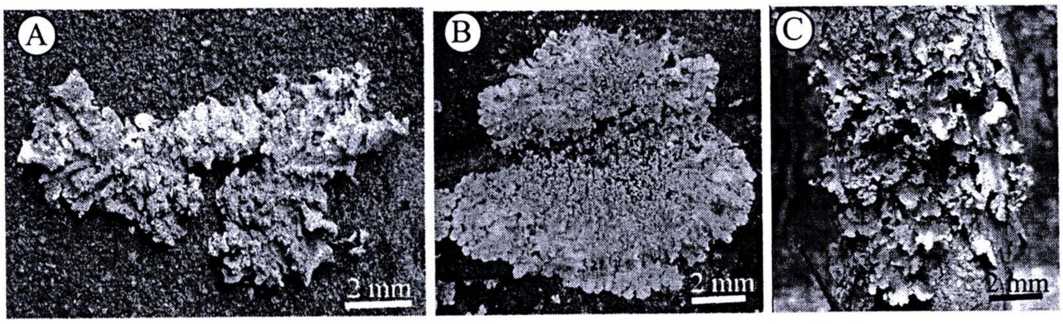

Figure 9 *P. sulphuratum* (A), *P. praesorediosum* (B) and *P. sancti-angelii* (C) with vegetative diaspores used for transplantation.

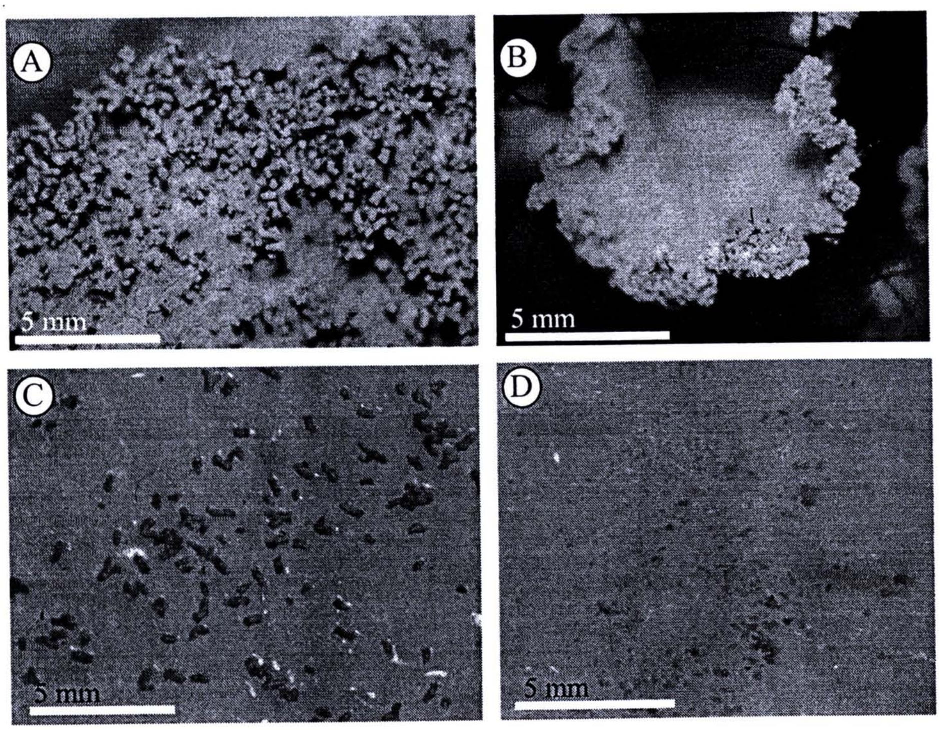

Figure 10 The isidia (A and C) and soredia (B and D) were adhered on DSGT.

Transplantation Design

Three main transplantation experiments were performed as follow:

1. Transplantation of site specific species among different ecosystem

2. Transplantation of vegetative propagules of lichens among different ecosystems including TRF, LMF, DEF and SF.

3. Transplantation of vegetative propagules of lichens on artificial substrates under various physical conditions in SF. This experiment consisted of five investigations.

These transplantation experiments were carried out as follow:

Transplantation of Site-Specific Species Among Different Ecosystems

This experiment was designed to test the adaptive capacity of lichens that originally grow in specific forest and then transplanted to other types of ecosystems. Thallus fragments of nine species specifically grow in LMF, TRF, SF were transplanted among each others. The transplanted lichens were attached on host trees in each forest. The experiment was performed during June 2005 to December 2008.

Host trees. A total of 12 host trees were used for lichen transplantations, three dominant trees were chosen from Tropical Rain Forest (TRF), Dry Evergreen Forest (DEF), Lower Montane Forest (LMF) and Secondary Forest (SF) of Khao Yai National Park (KYNP). These forests represented typical ecosystems influenced by different climatic factor. They also characterized the main ecosystems of Thailand, which are TRF-warm wet and dim light, SF-warm wet and sunny, DEF-warm humid and LMF-cool humid. The characters of all host trees in these forests were described in Table 1.

Table 1

Characters of Individual Host Trees in Tropical Rain Forest, Dry Evergreen Forest, Lower Montane Forest and Secondary Forest at KYNP

Trees code	Name	Height (m)	Bark surface	Canopy illumination
TRF 1	*Terminalia citrina*	30	smooth	moderate
TRF 2	*Malia sp.*	21	flaking	bright
TRF 3	Unkown1.	21	smooth, dusty	bright
DEF 1	Unkown2.	21	smooth	moderate
DEF 2	*Terminalia* sp.	24	smooth	dense
DEF 3	*Malia* sp.	21	flaking	bright
LMF 1	*Dacrydium elatum*	18	smooth, flaking	bright
LMF 2	*Schima wallichii*	15	smooth	bright
LMF 3	*Castanopsis* sp.	13	rough	moderate
SF 1	*Schima wallichii*	10	rough, flaking	moderate
SF 2	*Schima wallichii*	10	rough, flaking	bright
SF 3	*Syzygium* sp.	12	smooth, dust	bright

Transplanted lichens. Nine species of lichens grew in specific forest at LMF, TRF and SF were collected and transplanted among each others. A total of two thousand one hundred and fifty-five thallus fragments of these lichens were used. Numbers of thallus fragments of each species collected and transplanted to other forests were shown in Table 2 and Figure 11.

Of the transplanted lichens, two them *Relicina bstruse* and *Dirinaria picta* are common in TRF, DEF and SF (Ramkamheang University, Faculty of science, Department of biology, Lichen Research Unit, 2004), whilst others species were specific to particular sites (see Table 2). Thalli from warmer sites, e.g. TRF, SF and DEF, were transplanted to the cooler area at LMF, and *vice versa* (see Table 2). Eight species of lichens with green algal photobionts, prefer moderate light condition, were transplanted to canopies and mid trunks. *P. argyracea* contains blue-green algal photobiont, favors dim light, was transplanted to base of trees.

All thallus fragments were attached on substrate by nylon monofilaments (nylon fishing line) at the East aspect of tree trunk. Transplantation of site-specific species to SF was performed at the level of breast height on six host trees *Schima wallichii*. Rows of thallus fragments of site-specific species were alternately attached on tree trunk. Each row consisting of ten thallus fragments of the same species (see Figure 12).

Table 2

Lichens, Number of Samples and Sites of Collection and Transplantation of Site-Specific Species in Four Forest Types at KYPN During Jun 2005 to Dec 2008

Species	Collected from	No. of thalli transplanted to			
		TRF	DEF	SF	LMF
Hypotrachyna kingii	LMF	170	180	150	-
Parmelinella chozoubae	LMF	170	150	90	-
Heterodermia lepidota	LMF	170	120	180	-
Hypotrachyna osseoalba	LMF	110	90	-	-
Pseudocyphellaria argyracea	TRF	-	-	-	35
Relicina subconnivens	TRF	-	-	-	120
Relicina abstrusa	TRF	-	-	-	60
Parmotrema rubromarginatum	SF	-	-	-	180
Dirinaria picta	SF	-	-	-	180
Total		620	540	420	575
Grand Total		2155			

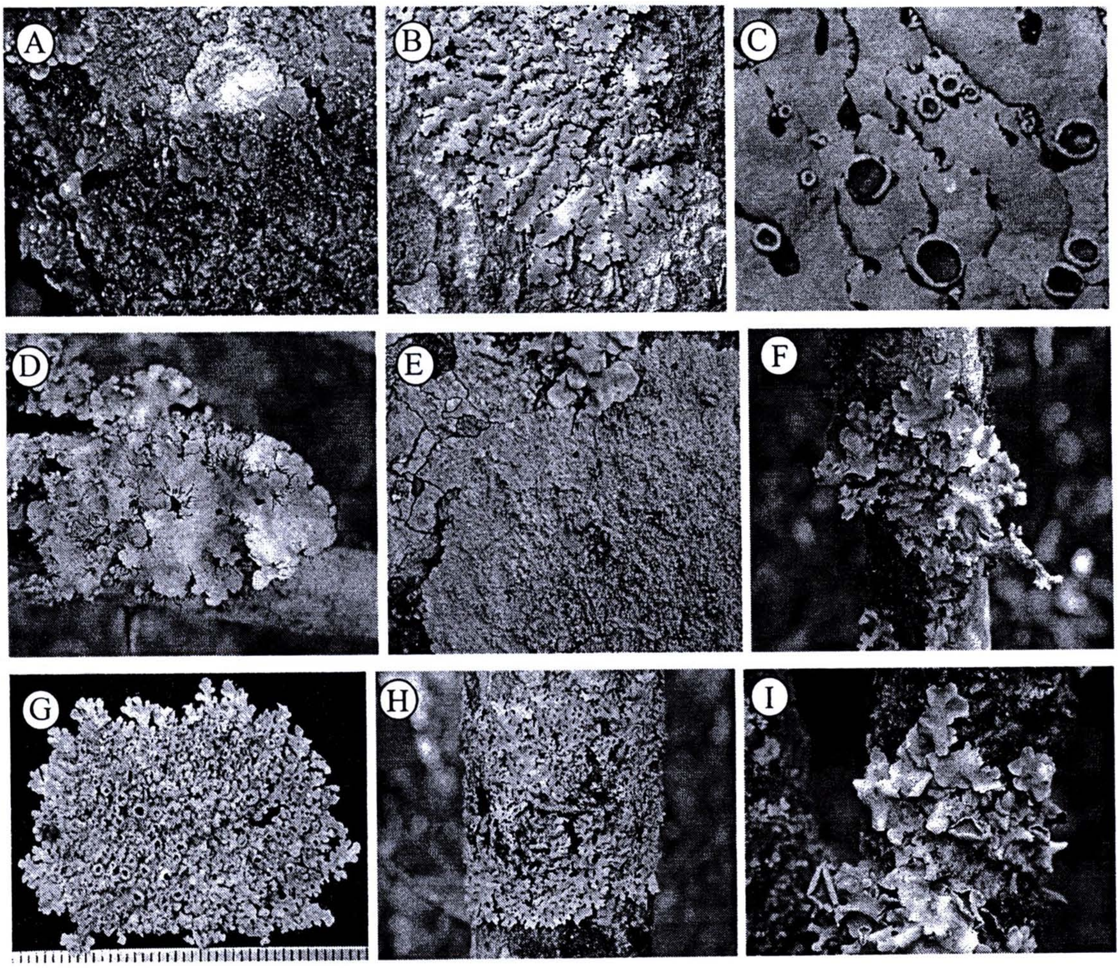

Figure 11 Site-specific species of lichens used for transplantation in various ecosystems.

Note. From researcher; *Pseudocyphellaria argyracea* (A), *Relicina abstrusa* (B) *Relicina subconnivens* (C), *Parmotrema rubromarginatum* (D), *Dirinaria picta* (E), *Hypotrachyna kingii* (F), *Heterodermia lepidota* (G), *Hypotrachyna osseoalba* (H) and *Parmelinella chozoubae* (I).

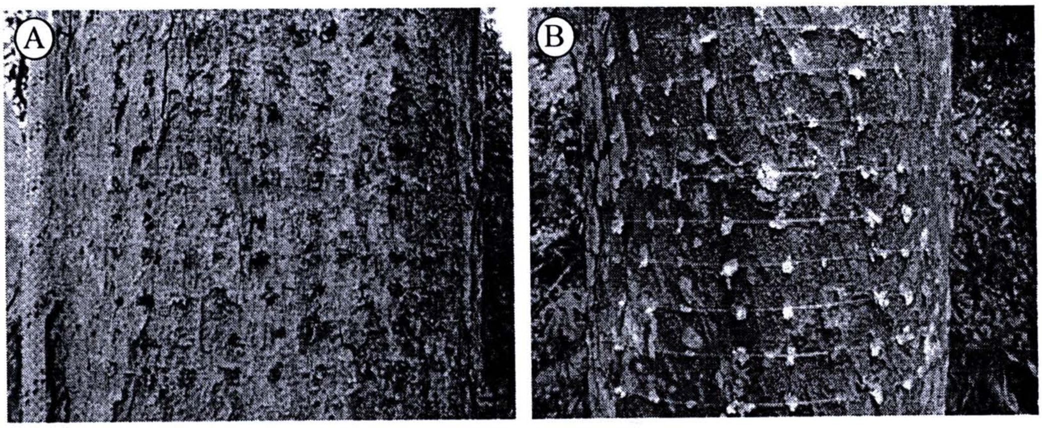

Figure 12 Thallus fragments of site-specific species attached on host trees at canopy in TRF (A) and mid-trunk in LMF (B).

Transplantation of Vegetative Propagules of Lichens in Various Ecosystems

This experiment was set up to test the ability to establish and survive of vegetative propagules including thallus fragments, isidia and soredia after transplanted to different ecosystems. It intended to find the best kind of vegetative propagule appropriate for transplantation in particular ecosystem.

Transplantation of thallus fragments to various ecosystems. Thalli of *P. tinctorum* were collected from SF during October to December 2003. Five hundred and fifty two thallus fragments were transplanted to the same host trees (see Table 1) used in previous experiment at TRF, DEF, LMF and SF. Thallus fragments were transplanted by fasten on bark of host tree with nylon monofilament (see Figure 13A and C) . Transplantation were made in the selected forest as follow;

1. Tropical Rain Forest (14°26.254' N 101°22.043' E): One hundred and ninety two thallus fragments were transplanted to three host trees in this forest. The host trees were TRF1 (*Terminalia citrina*), TRF2 (*Malia* sp.) and

TRF3 (Unknown-1). These trees hosted 80, 56 and 56 thallus fragments respectively. Transplantation was performed during October 2003 to November 2003.

2. Dry Evergreen Forest (14°27.825' N 101°22.196' E): One hundred and eighty thallus fragments were transplanted to this forest. The three host trees consisting of DEF1 (unknown-2), DEF2 (*Terminalia* sp.) and DEF3 (*Malia* sp.) Numbers of thallus fragments transplanted to these hosts were 56, 64 and 60 respectively. Transplantation was performed during October to November 2003.

3. Lower Montane Forest (14°24.047' N 101°24.275' E): One hundred and twenty thallus fragments were transplanted on three host trees included LMF1 (*Dacrydium elatum*), LMF2 (*Schima wallichii*) and LMF3 (*Castanopsis* sp.). Numbers of thalli transplanted on each host tree were 48, 36 and 36. Transplantation was done during November to December 2003.

4. Secondary Forest (14°25.272' N 101°22.400' E): Sixty thallus fragments were transplanted on SF2 (*Schima wallichii*) and SF3 (*Syzygium* sp.). Twenty eight and 32 of thallus fragments were transplanted to these trees during November to December 2003.

Transplanting gear. Tree-climbing gear with robe support was used for transferring lichen materials to various heights of host trees. Tree trunks were marked at every 1.5 meter from the bases to the canopies (see Figure 13C). Thallus fragments were fixed on barks at the North (N), East (E), South (S), and West (W) by using 0.5 mm diameter of nylon monofilaments.

Transplantation of isidia and soredia to various ecosystens. Isidia of *Parmotrema tinctorum* and soredia of *P. praesorediosum* collected from SF during November to December 2003 were spread on DSGT, which was used as material for supporting isidia and soredia. The DSGT were covered by surgical gauze in order to protect the vegetative propagules against wind and rain (see Figure 13B). These substrates loaded with diaspores were attached on tree trunks at 10 cm. above and below the level of thallus fragments transplantation. The isidia substrates were attached above the lines of thallus fragment, whereas those of the soredia were placed below. Paper staple was used to fix the substrates on bark of host trees (Kon et al, 2003; Scheidegger, 1995) at the four aspects of orientation as described earlier in thallus fragment transplantation (see Figure 13B and C).

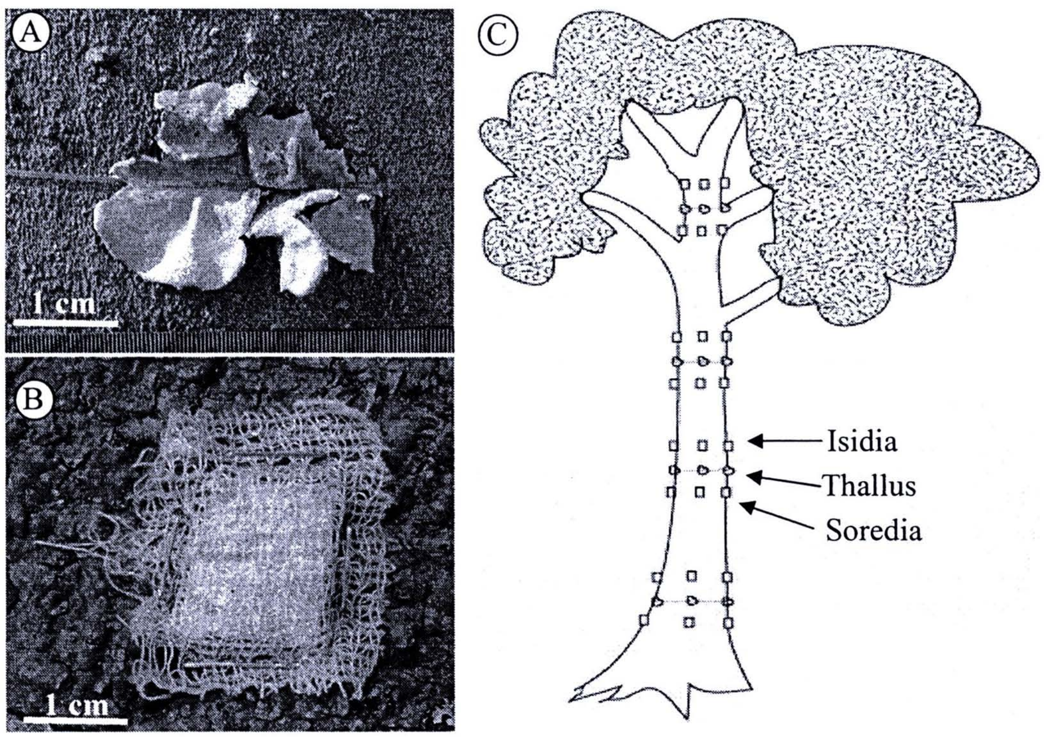

Figure 13 The thallus fragments (A), isidia and soredia (B) transplanted to various levels on hosted trees (C).

Transplantation Vegetative Propagules of Lichens on Artificial Substrates

This experiment was designed to find suitable substrates that lichens can grow in diverse physical condition. It consisted of the following experiments:

(1) Transplantation of vegetative propagules of lichens on artificial substrates in different forests. Thallus fragments and isidia of *P. tinctorum* and soredia of *P. sancti-angelii* (see Figure 13) collected from SF in June 2005 were transplanted on artificial substrates including ceramic tiles, rubber tile, wood board, galvanized iron, concrete block and brown glass-bottle. These substrates were installed at the LMF, SF and TRF. Twenty-five each of thallus fragments, isidia and soredia, were adhered on Double Side Glue Tape (DSGT) and attached on these substrates (see Figure 14). The substrates were submerged in water for 24 hours before using to remove impurities. They were then hanging vertically by two parallel wires at 1.5 meters above the ground at LMF and SF, whereas scaffold was used at TRF. The experiment was performed during June 2005 to December 2008.

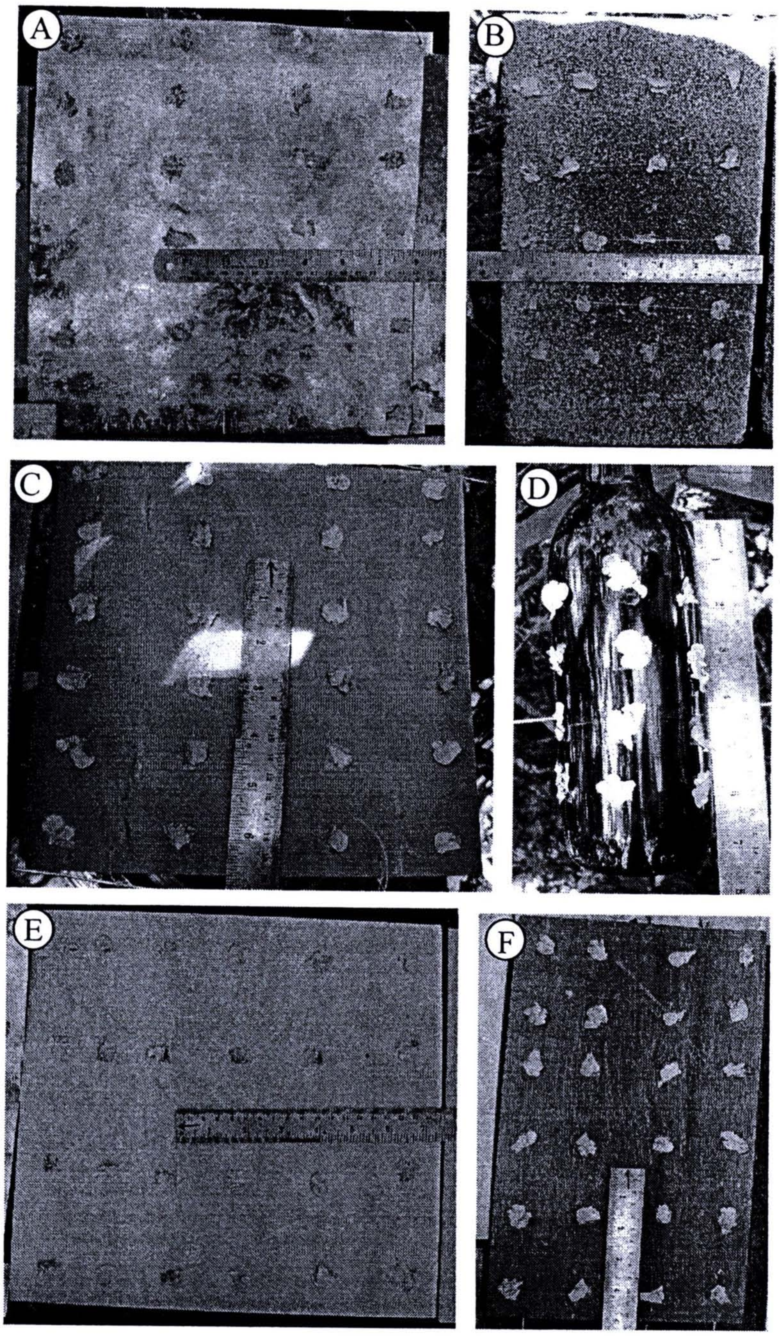

Figure 14 Thallus fragments adhered on various artificial substrates.

Note. From researcher; thallus fragments of *P. tinctorum* on artificial substrates; galvanized iron (A), concrete block (B), rubber tile (C), brown glass-bottle (D), ceramic tile (E) and wood board (F).

(2) Transplantation of thallus fragments on polycarbonate plates under canopy of SF. Pong-thong-kawng (Nong King) in SF was selected as the transplanted area (14°25.272' N 101°22.400' E). The experimental plots of 4x4 m^2 were made for lichen transplanted under canopy of SF in this area. Thallus fragments of ca. 2x2 cm^2 from marginal lobe of *P. tinctorum* collected from the nearly transplanted areas in SF were used. One hundred and seventeen of them were adhered on 117 polycarbonate plates by nylon monofilaments (see Figure 15A). These substrates were immersed in water to remove impurities for 24 hour. Transplantation was performed by fixing substrates over two parallel wires hanging about 1.5 meter over the ground under natural canopy shading during June 2004.

(3) Transplantation of thallus fragments on ceramic tiles and plastic nets under shading net in SF. This experiment was located nearby the experimental plot described in (2). This plot was covered by green shading nets at 0.5 m above the transplanted substrates. Therefore it was shaded by canopy and man-made shading net. Thallus fragments at the marginal lobe of approximately the same size (ca. 2x2 cm^2) from *P. tinctorum* collected from the nearly transplanted areas in SF were used for transplantation. One hundred sixteen and 114 thallus fragments were fixed on 116 ceramic tiles and 114 black plastic nets respectively by nylon monofilaments. The experiment was carried out during May 2005 (see Figure 15B and C).

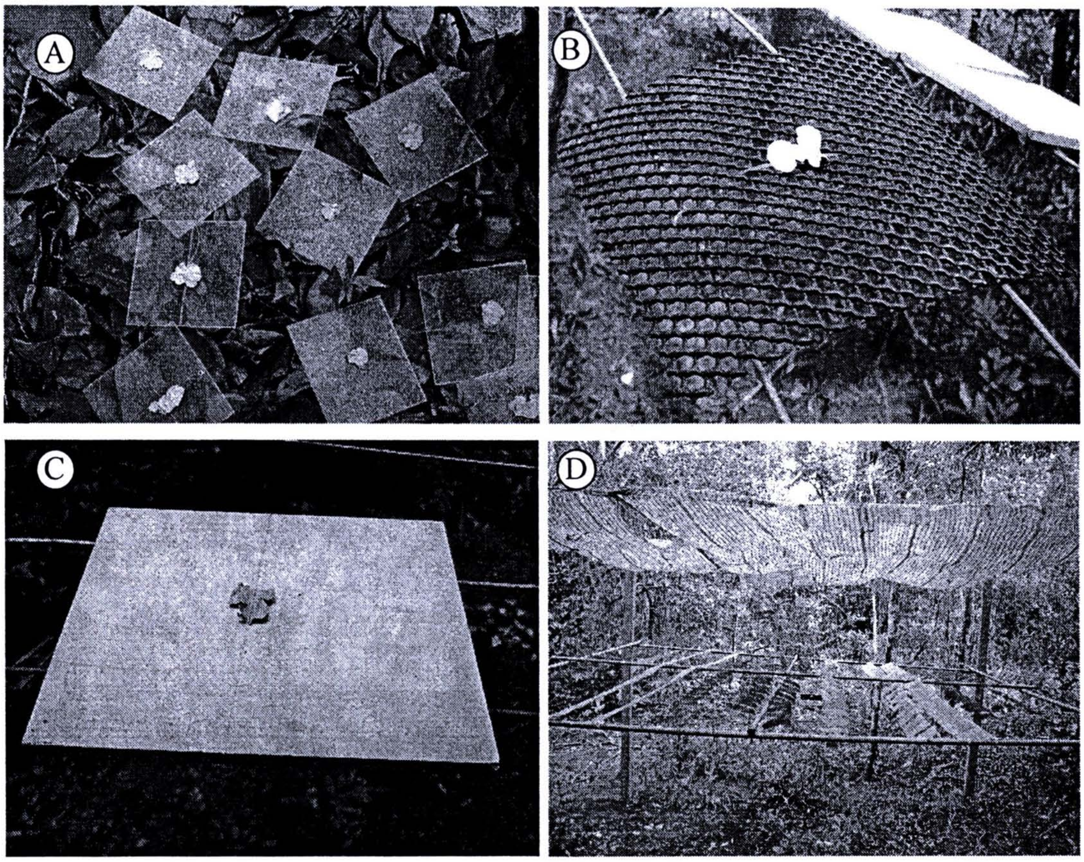

Figure 15 Thallus fragments over artificial substrates, polycarbonate plates (A), plastic nets (B) and ceramic tiles (C). Installation of transplanted materials in Secondary forest (D) in May 2005.

(4) Transplantation of isidia on nylon net at different angel of inclination under shading net. Isidia of *P. sulphuratum* collected from SF were sown on thirty DSGT (see Figure 9A). They were then adhered in square bags of nylon net (mosquito plastic screen, see Figure 16) and transplanted to the same experimental plot as (3), under shading net in SF (see Figure 15D). The substrates were fixed on two parallel wires, as described earlier, by facing to the East on horizontal (0^{o}), vertical (90^{o}) and 45^{o} slopes. The experiment was performed in June 2005.

(5) Transplantation of isidia and soredia on nylon net at 45° inclination under shading net in SF. *I*sidia of *P. tinctorum* and soredia of *P. sancti-angilii* were spread on of DSGT and enclosed by square bags of nylon net (see Figure 16). Fifty pieces of each soredia and soredia samples were fixed on parallels nylon ropes (see Figure 15D), making 45° slope. The transplantation was conducted under shading net at the same experimental plot as 3.3 during April 2006.

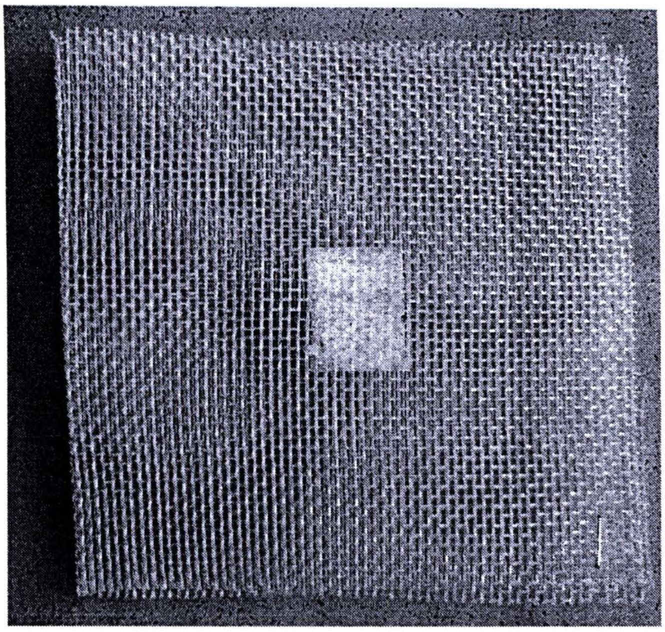

Figure 16 The transplant material, DSGT, holding isidia or soredia enclosed by nylon bag.

Note. From researcher; isidia was from *P. tinctorum* and *P. sulphuratum*, and soredia was from *P. santi-angelii*

Measurement of Microclimate in the Transplanted Sites

Microclimate was measured exactly at the habitat of lichen transplantation on one of the host trees to represent growing condition of lichens in each forest. Simultaneously, microclimate of two forest types were measured and recorded by two data loggers. Microclimatic factor including light intensity, air temperature and relative humidity were recorded. Three light sensors (Li-cor 190SB) were installed on tree base, mid-trunk and canopy. Two temperature and humidity sensors (HMP 50, Vaisala, Inc.) were installed at tree base and canopy (see Figure 17). Data from all sensors were recorded and stored with 21x data logger (Campbell Scientific, Logan UT, USA) and Li-1400 data logger (Li-Cor, Lincoln, NE). Measurements were done every second and the averages were taken and recorded every 5 minutes interval for 24 hours or up to seven days continuously. Some data was shown in Figure 18.

Figure 17 Data logger Li1400 and sensor installed on trees trunk.

Note. From LI-COR Biosciences "http://www.licor.com/env/products/light/datalogger.jsp". Quantum sensor (Li-190 SB), Temperature and (HMP50) Relative Humidity sensor (A), and Data logger (Licor-1400) (B). Installation of sensor at a host tree in SF (C).

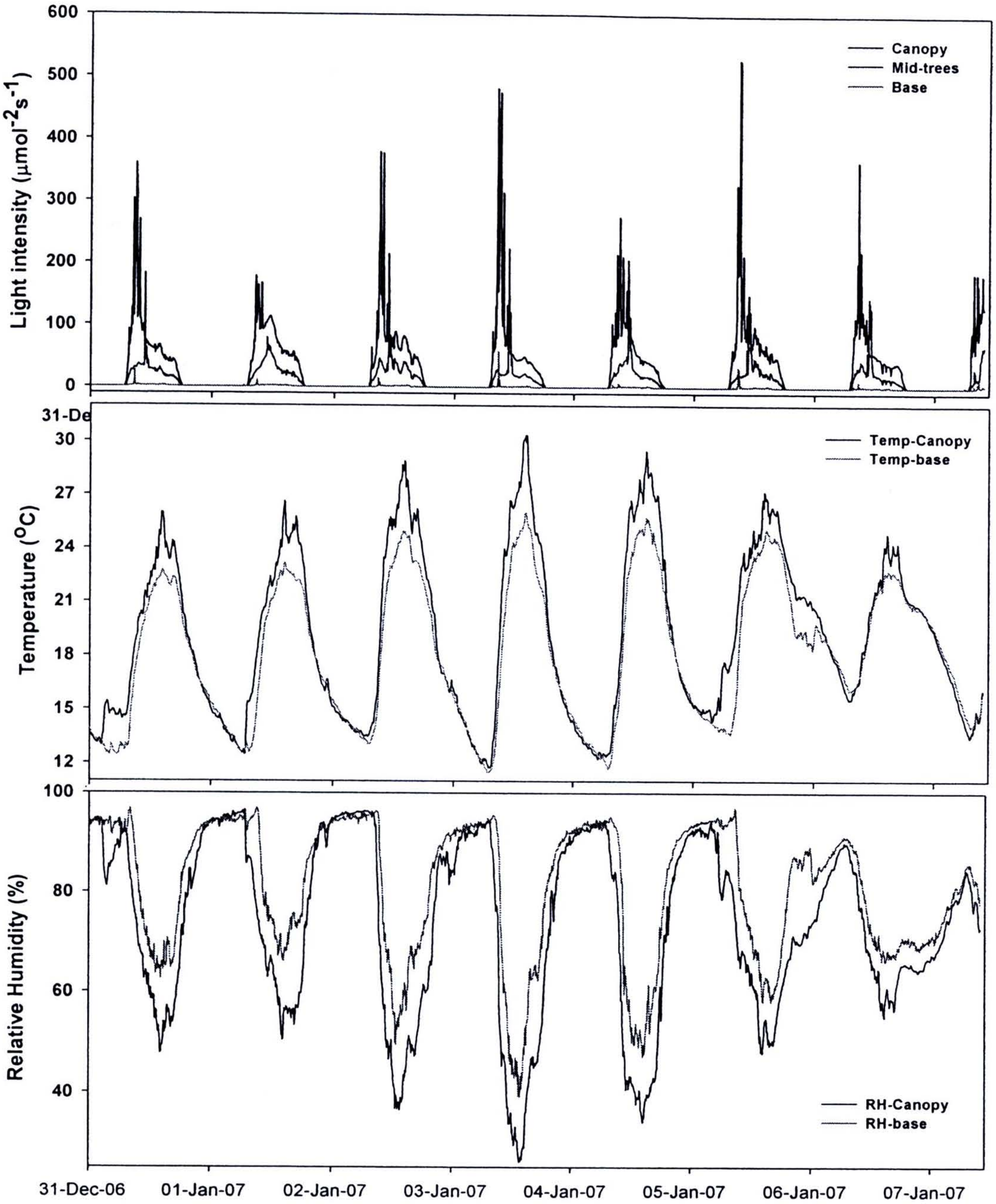

Figure 18 Microclimatic data of lichen habitats along tree trunk at KYNP.

Note. Form researcher: Light intensity, temperature and relative humidity recorded from TRF during 31 December 2006 to 7 January 2007 (— = base trees, — = Mid-trunk, — = Canopy).

Data Collections

Survival of Thallus Fragments

Mortality and survival of the transplanted thallus fragments were counted every 2, 4, 6, 8, 10, 15, 20, 25, and 30 months. The data were stored and managed in Excel for further analysis.

Growth Rate of Thallus Fragments

Growth rate were collected by drawing edges to thalli on overlying transparent sheets every six months according to method described by (Nimitr Osathanon, 2002). These transparent sheets were scanned. Areas and diameters of lichen thalli were calculated by Zeiss *AxioVision LE Rel. 4.1* software (Carl Zeiss MicroImaging, Inc., see Figure 19). Growth rate (mm/month) was calculated by equation 1.1 (Nimitr Osathanon 2002).

$$\text{Growth rate (mm/month)} = \frac{D_2 - D_1}{T_2 - T_1} \qquad 1.1$$

when D_1 = Diameter at the beginning of measurement

D_2 = Diameter at the last measurement

T = Measurement at time 1 (T_1) and time 2 (T_2)

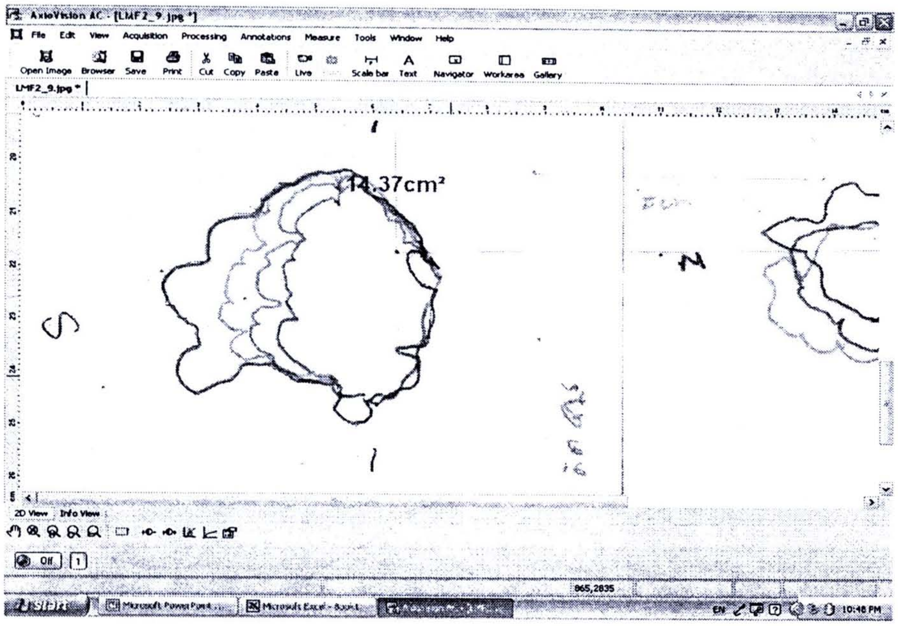

Figure 19 Outlines of thallus expansion drew on transparent sheet at time intervals after transplantation.

Note. From researcher; Thallus outlines viewed under Zeiss AxioVision LE Rel. 4.1 software. The outlines were scanned and used to calculated changes in areas and diameters, of which growth of lichens was estimated.

Survival and Growth of Diaspores

Development of isidia and soredia were observed and photographed every 6 months; Survival, change and growth of these diaspores were counted and calculated as percentage (%). Occasionally, diaspores attach on DSGT was collected for diaspore photographed under stereomicroscope.

Statistical Analysis

The correlations of microclimates were evaluated with growth and survival of vegetative propagule after transplanted during three years by using ANOVA. The analyses were shown by Filled Contour Plots graphs that compared different thallus fragments grew in various transplanted areas. Nonlinear regression analysis was used to find relationship of growth rates of vegetative propagules at various heights of the host trees. Differences in survival and growth of thallus fragments and diaspores in various ecosystems were test by using One-way ANOVA. The data analysis and graphic representation were accomplished by using Sigmaplot 11.0 software (Systat Software Inc.).