CHAPTER 2

LITERATURE REVIEW

Life of Lichens

Distribution

A lichen is a symbiotic organism composing of a fungus and an alga. Lichen grows on various substrates such as soil, rock, bark, leave and animal carapaces (Seaward, 2008, p. 274). They are poikilohydric organism and thus can grow in extreme habitats using water and materials from the atmosphere. Each species has different strategies to preserve water in the thallus. Water content in thallus is important for lichens. Several species depended on relative humidity and fogs.

Lichens disperse by 2 main methods. First, air-born fungal spores can germinate when landing into suitable habitat. They incorporate algal partners and grow into lichen thalli. Second, vegetative propagules, such as isidia, soredia and thallus fragments, which consist of both fungi and algae, have immediate ability to grow into lichens. After having been dispersed by wind and water (Ahmadjian, 1993; Armstrong, 1990a; Hilmo & Ott, 2002; Ott, Kappen, & Sancho, 2004; Scheidegger, 1995; Scheidegger, Frey, & Zoller, 1995; Stocker-Wörgötter & Türk, 1988), these propagules can also adhere to animals into far another region (Heinken, 1999; Seaward, 2008, p. 277). The

distribution of lichen vegetative propagules on vertical tree trunk and rock surface are assisted by rain droplets (Armstrong, 1990a). Wind speed at around 2 ms^{-1} is able to move soredia from dry thallus to elsewhere (Armstrong, 1992; Seaward, 2008, p. 276). Öckinger, Niklasson, and Nilsson (2005) reported that the distributing distance of *Lobaria pulmonaria* was up to 75 m by wind. Also, Werth et al. (2006) found that the spores and diaspores of *L. pulmonaria* could be carried for more than 200 m.

Recently, studies on processes of lichen distributions found that tree height also influence lichen dispersal (Antoin & McCune, 2004; Eversman, Johson, & Gustafson, 1987; Hale, 1952; 1965; Moning et al., 2009; Sipman & Aptroot, 2001). For example, Wetchasart Polyaim (2005) reported that lichen diversity on canopy was higher than on mid trunk and tree base of *Castanopsis acuminatissima* and *Dipterocarpus gracilis* in tropical rain forest at KYNP. This was because the influence of microclimate (temperature, humidity and light intensity) affected lichen growth differently at different tree height and in different host trees. Especially, canopy of tropical rain forest extremely decreases light penetration to forest ground. Figure 1 illustrates that at the ground level, remaining light intensity was only about 0.5-1 percent of full-sun irradiance (Lüttge, 2008, p. 86). According to Sillett and Antoine (2004), different canopy characteristics can affect selective habitats of lichens. For example, some lichen species, such as *Leptogium* and *Stigta*, could survive only under dense canopy which allows low light intensity penetration to the ground.

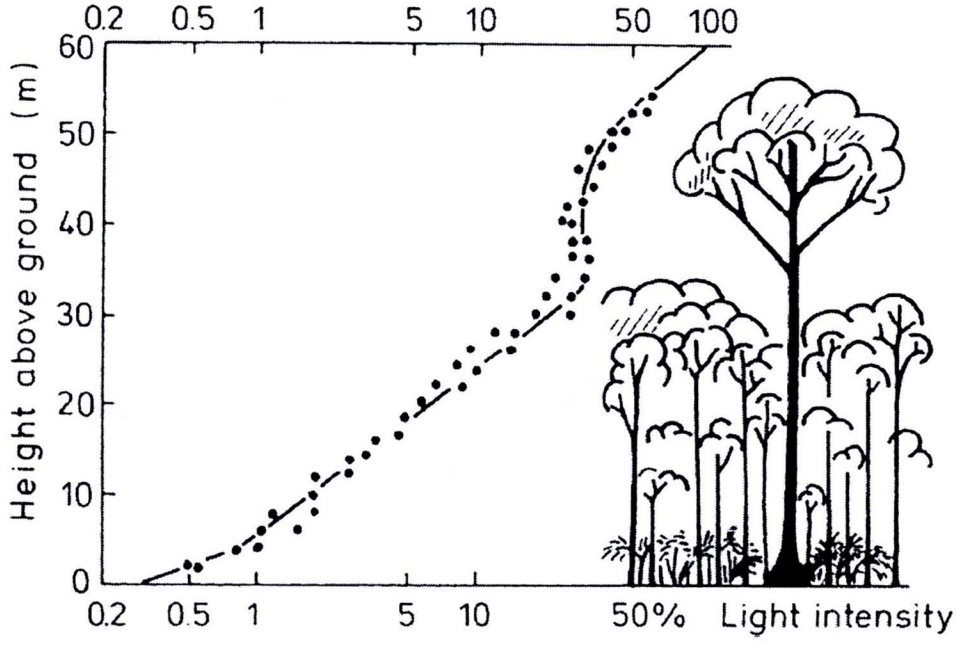

Figure 1 Light penetration through the canopy of a tropical forest.

Note. From *Physiological Ecology of Tropical Plants* (p. 86), by Ulrich Lüttge, 2008, Berlin: Springer.

Establishment

After reproductive structures of lichens are distributed to new substrata in suitable habitats (Armstrong, 1988), propagules would grow into mature thalli. Thereby, suitable environmental factors and substrates are pivotal to successful lichen germination and establishment into mature thalli (Green, Nash, & Lange, 2008, pp. 152-181; Seaward, 2008, pp. 274-298). In order to develop into a thallus, a germinated fungal spore needs to match with its specific algal partner. On the contrary, vegetative propagules could directly adhere and grow on the surface. However, the establishment of both types of the reproductive structures could be hindered by the impact of raindrops,

moisture and wet and dry alternation (Scheidegger, 1995; Scheidegger & Werth, 2009; Zoller, Frey, & Scheidegger, 2000).

Growth

Lichen could synthesize its own nutrition. Algae, the photosynthetic partner, make up ca. 10% of total lichen biomass (Green et al., 2008, p. 152). The photosynthetic processes are very important for lichen growth. Its efficiency was limited by light quantity and thallus water content reserve capacity. The water content in lichens thallus decreases when air moisture decreases, i.e. in the late morning or afternoon. This leads to short periods of water availability and thus efficient photosynthetic process (Ahmadjian, 1973; Armstrong, 1974; Nimitr Osathanon, 2002; Palmqvist, Dahlman, Jonsson, & Nash, 2008). Although these processes occur dairy, they occur only within a limited period of time. This limits lichen growth in nature. Different species have different ability in thallus water reserve and light usage and therefore can grow at different rates.

Rogerson, Evans, and McCoy (1986) studied growth rate of lichens by measuring an increase in thallus sizes. They found that *Rhizocarpon* and *Alectoria miniscula* had very slow growth rates at around 0.10 and 0.54 mm/yr respectively at the mountains south of the Arctic (Low-arctic Mountain). Lange, Pfanz, Kilian, and Meyer (1990) reported that the crustose and foliose lichens in the temperate had the growth rates of 0.03 and 4.82 mm/yr respectively. Recently, Nimitr Osathanon (2002), who studied the

growth rate of lichens in the tropic forests in KYNP, found that the average growth rate of foliose and crustose were 6.4 mm/yr (up to approximately 20.4 mm/yr) and 1.3 mm/yr (maximum 12 mm/yr) respectively. His results indicated that lichens in the tropic grow faster during rainy seasons due to high moisture and rain in more than 6 months. In addition, Ahmadjian (1973, pp. 565-579) reported that under high moisture or rain, the photosynthetic process of lichens could be reactivated during the daytime.

Survival

Lichens could survive under appropriate environment where are associated to sufficed of climate factor and nutrient source lead to lichen growth and net photosynthesis occurring (Palmqvist et al., 2008, pp. 182-215). Some lichens could resist environmental stress better than the others. For example, crustose lichens have been estimated to survive well over 1000 years although they are growing on rock surface under extreme environment (Nash, 2008a, pp. 4-5). The survival of lichens population in nature depends on several factors. For example, Armstrong (1990b) found that the microtopography of tree bark influenced soredia establishment and survival. Reproductive propagules did not survive on smooth bark surface as well as on rough bark surface, especially in highly competitive habitats (Mikhailova & Scheidegger, 2001; Seaward, 2008). Furthermore, lichen thalli are soft under high humidity. This made it easier for the thalli for insect and snail grazing (Gauslaa, Holien, Ohlson, & Solhøy, 2006a). Currently, air pollution is posing

a serious problem for lichen thallus development and survival. For instance, Mikhailova (2007) found that air pollution affected the development of soredia more than that of thalli and also limited soredia dispersal in polluted areas (Nash, 2008b; Schuster, Ott, & Jahns, 1985).

In addition, forest management and changes in ecosystems greatly affected lichen survival. According to Scheidegger et al. (2000) model on patterns of lichen survival and extinction in well-preserved forests and poorly-managed forests, lichens survival in poorly-managed forests will continually decline in the next 200 years. Particularly, lichens in some areas would be more severely affected by changes in their habitats and could become endangered (Scheidegger & Werth, 2009).

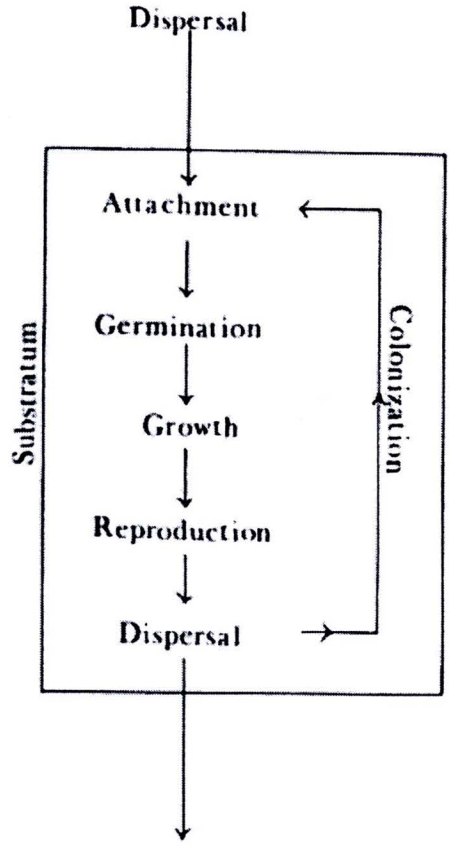

Figure 2 Stages in life cycle of a lichen population colonizing a substrate.

Note. From "Substrate colonization, growth and competition" by R. A. Armstrong, 1988, *CRC Handbook of Lichenology, volume II*, p. 4.

Lichen Transplantation

The lichens transplantation in nature is used to increase lichen propagation and productivity. Successful transplantation depends on environmental factors that affect lichen survival, establishment, development, adaptation and growth. A number of lichen transplantation methodologies, including the use of different reproductive structures, have been developed and applied in the temperate for conservation reasons (Armstrong, 1990a; Gilbert, 1991; McCune et al., 1996). However, there has never been a study on lichen transplantation for conversation and productivity augmentation in Thailand. There are two relevant studies that worth being mentioned though. Kansri Boonpragob (1993) studied the influence of air pollution on chlorophyll degradation by transplanting lichen fragments from KYPN to new urban and suburban locality. Recently, Kittiya Jiathanakul (2005) studied the accumulation of heavy metals in lichens by transplanting *Parmotrema tinctorum* from Khao Yai National Park into Ramkhamhaeng University. They found that lichens accumulate heavy metals in their thalli since being transplanted to the city.

Transplantation Techniques

Transplantation Using Reproductive Structures

Lichens produced numerous spores in apothecia for dispersal into remote areas. Therefore, lichen spores can be cultured into thalli in laboratory. Fungal spores and photobiont cells were cultured separately under artificial conditions and were reintroduced for both partners to develop into symbiotic lichen thalli (Ahmadjian, 1973, pp. 565-579; Stocker-Wörgötter & Hager; 2008, pp. 353-363). For example, Stocker-Wörgötter and Türk (1991) cultured new thalli by using the spores of *Peltigera praetextata*, coupled them with their algal partner, then transplanted developing juvenile thalli on sterile soil under the trees in natural conditions. *P. praetextata* cultivated could grow up to 15-30 centimeters in diameter. Moreover, they could survive for more than three years, producing soredia for propagation.

Transplantation Using Vegetative Propagules

Thallus fragments transplantation. Lichen transplantation by thallus fragments is most favored because they consist of both algal and fungal partners and could grow readily and rapidly. However, this method needs a large quantity of lichen thalli as lichen source. Only healthy lichen thalli are selected for transplantation as thallus sizes and ages greatly affect their adaptation and growth in transplantation sites (Gauslaa, Lie, Solhaug, & Ohlson, 2006b). Brodo (1961) designed a method for transplantation by

removing thalli of *Parmelia caperata* along with their bark substrates and transplanting the undamaged bark disks on other trees nearby, where the same species of lichen had been found growing, to study the influence of air quality. Afterward, Armstrong (1977) studied lichen growth rates and patterns by transplanting the thallus fragments of *Physcia orbicularis*, *Parmelia conspersa*, *P. glabratula ssp. Fuliginosa* and *P. saxatilis*. The initial diameters of these thallus fragments were 2-13 centimeters. They were transplanted on the left side of wood boards that were placed on rock surface facing southeast and northwest. It was found that transplantation direction affected lichen growth owing to the influence of microclimate, especially the higher light intensity and temperature in the southeast than in the northwest. Gilbert (1991) developed a thallus fragment fixation approach by using epoxy resin (Araldite) to adhere *Lobaria amplissima* thallus fragments on substrates. The fragments were fixed on the eastern side of the bark of *Quereus petraea* and *Acer pseudoplatanus* trees. One year after having been transplanted, 70 percent of thallus still remained and were growing at 2.2 mm/yr. Two year after having been transplanted, their growth increased to approximately 4-5 mm/yr. Lastly, after 10 years, the thallus sizes had increased to about 3-5 times their original sizes.

Kon, Mineta, and Kashiwadani (2003) transplanted 7x7 mm^2 thallus fragments of *Parmotrema tinctorum* into nearby natural sites, in which the species had been found growing on the trunks of *Cryptomeria japonica* D. Don. The thallus fragments were covered with 2 x 2 cm^2 nylon mesh and stapled at the corners to hold the fragments on tree bark. In four months, the

samples had morphological changes and increase in size up to 69 mm^2. In 15 months, the sizes increased to 83 mm^2. In addition, they produced numerous juvenile isidia on the upper surface and rhizines on the blackish lower surface. The transplantation of these thallus fragments of *P. tinctorum* using nylon mesh was successful.

Studies of lichen transplantation largely focused on measurable environmental factors that influenced lichen growth. Sillett (1994) transplanted thalli of *Lobaria oregana* and *Pseudocyphellalia rainierensis* which were collected from tree barks at the canopy in the heart of an old-growth forest. The samples were transplanted on canopy bark at the edge between the old growth and the clear-cut areas. Also, thallus fragments collected from the forest edge were transplanted deep in the middle of the forest. The thallus fragments were hanged from tree branches with nylon ropes. Within one year, the *L. oregana* transplanted in the central forest areas had higher growth rates than those transplanted in the forest edge. *P. rainierensis* transplanted in the central forest had no growth. However, when removed from the central forest and transplanted back into the edge forests, *P. rainierensis* had higher growth rates. Fragments of *L. oregana* had higher average growth rates than *P. rainierensis* because they were adapted to the higher humidity in the canopies of central forest (Armstrong, 1993) while high light intensity and ventilation at the forest edge dried their thalli rapidly (Esseen, 2006; Esseen & Renhorn, 1998). On the contrary, *P. rainierensis* was adapted to the microclimate of forest edge and therefore had higher growth rates at the forest edge but lower growth rates in central forest. Sillett and

McCune (1998) studied the survival and growth of thallus fragments of both *L. oregana* and *P. rainiarensis*. The prior was a common species and the latter was a rare species in the study areas. Their fragments were adhered on canopy branches, whose surfaces were divided into two groups: uncovered and covered by moss, in four coniferous forests, including old growth forest (400-700 yr), mature forest (140-150 yr), young forest (35-40 yr), and clear-cut areas. Transplanted *L. oregana* had approximately 4 times higher growth rates in old-growth forest than in the clear-cut. The mortality rates in the clear-cut were also about 10 times higher than in the old growth forest. The rates of growth and mortality of *P. rainiarensis* were similar to those of *L. oregano*. *P. rainiarensis* could not survive in the clear-cut forest because this area had extreme climate. In addition, *P. rainiarensis* had higher growth rates on moss surface than other surfaces. This was due to greater moisture accumulation and permeation into lichens for extended photosynthetic time. Caldiz (2004) studied the influence of seasons on growth rates of thallus fragments of *Pseudocyphellaria berberina.* The study also compared 2 methods of transplantation: pendant transplantation (PT), where the fragments were freely suspended by nylon rope between two *N. dombeyi* trees, and tree transplants (TT), where the thallus fragments were attached on tree trunks. Two year after having been transplanted, the PT samples had higher growth rates than TT ones while the TTs had higher survival percentages than the PTs, accounting for 81% and 59% respectively. However, the lower survival rate of PT was due to breakage of branches which destroyed many transplanted fragments during the winter season in 2002. There was no significant difference in

growth percentages of both treatments, accounting for about 12.6% increased growth. The highest growth was observed in winter, autumn and spring, subsequently. In conclusion, the influence of higher humidity and lower temperature could promote lichen growth. This study suggested a number of factors which affected transplanted lichen growth, such as changing canopy conditions (Boucher & Nash, 1990; Muir, Shirazi, & Patrie, 1997; Renhorn & Esseen, 1996; Sillett, 1994). In addition, seasonal changes lead to changes in microclimate and consequently attribute to uneven annual growth rate of transplanted lichens (Brodo, 1961).

Antoine and McCune (2004) transplanted thallus of lichens *Lobaria oregana*, *Lobaria pulmonaria*, *Letharia vulpina* and *Usnea scabrata* to investigate the influence of canopy position, *i.e.* the height of canopy, on the prevalence of the four lichen species and to study the association between lichen growth rates and vertical abundance in nature. The findings in this study could be summarized as follow.

In an experiment to study the effect of canopy height on an increase in the biomass of *L. oregana* and *Usnea*, the lichen samples were hanged in gondolas between light gaps in the canopy. After being transplanted, it was found that the growth rates of *U. scabrata* increased by 13, 25, 44, 61 and 61, in relation to an increase in canopy heights of 3, 13, 23, 33 and 43m, respectively. *L. oregano* grew at 13 m high from the ground. The understory level, about 3 meters high from the ground, was not suitable for many lichens species.

In the second set of experiment, the researchers installed 50 thallus fragments of pendant lichens *L. oregana* and *L. vulpina* on the crown of a single large Douglas-fir. The results also showed that the pendent lichens had increased growth rates in relation to tree height. The maximum growth rate was observed at mid-canopy (39-48m), while lichens at the height of 3m or less had lower growth rates.

In their last set of experiment, Antoine and McCune (2004) hanged the thallus fragments of *L. pulmonaria* on the branches which extended from north to south. The results showed canopy height had a greater influence on *L. vulpine* and *U. scabrata* growth rates. Certainly, the highest growth rates of both lichens were observed at the mid-canopy to the upper canopy. On the other hand, *L. oregano* and *L. pulmonaria* had highest growth rates at mid-canopy as they required low light intensity and could not withstand extreme climate, *e.g.* rapid wet–dry period shift, higher wind and light intensity, which was present at higher canopy.

Another interesting transplantation methodology was developed by Gauslaa et al. (2006) who studied the growth and ecophysiological acclimation of the foliose lichen *L. pulmonaria* in forests with contrasting light climates. They transplanted lichen thalli on square frame holders (15.9×15.9 cm^2) installed in 3 different forests: (1) evenly-aged young and canopy stands, (2) old forest with tree gaps, (3) open clear-cut areas with sparse regeneration of scattered small trees (<2 m tall), with no self-shading of branches. A group of samples were sprayed with 16 ml deionized water, equivalent to 0.63 mm rainfall sufficient to hydrate the lichen without

excessive dripping. The second group of lichen samples received the same spraying regime but with added nutrients equivalent to 0.336 mg N in each 16 ml spraying. The third group was the control and was not sprayed. The result revealed that the additional moisture given to the transplanted lichens during the experiment did not increase their growth because the experiments were done during the rainy season. Therefore, a slight increase in thallus areas could be attributed to added nutrients rather than the extra moisture. The lichens which were transplanted in the clear-cut forest had the highest increase in dry weight (23.1%). The increase in dry weight of the samples in the old forest and the dark young forest were 16.0 and 8.3%, respectively. The study also reported some of internal and external factors that were related to growth. For example, an expose to high light intensity in clear-cut forest was considered to be related to thallus thinning and a decrease in total chlorophyll content (Campbell, Hurry, Clarke, Gustafsson & Öquist, 1998; Gauslaa & Solhaug, 1999). In addition, transplanted thalli were densely covered with soredia or isidia which appeared to reduce the thallus growth rates.

Webster and Brown (1997) studied transplantation of terrestrial lichens *Peltigera canina* on soil in flowerpots, some of which being immersed in water to increase moisture. After transplanted, all thalli could grow from 0 to 6.4 cm in the first year with the longest growth of 9.3 cm after 600 days of transplantation. This experiment suggested that additional moisture in the soil increased lichen growth rates because lichen had prolonged hydration time. (Palmqvist, et al., 2008).

Lichen growth forms as the factors which influence transplanted lichen growth were also studied. McCune et al. (1996) studied the effects of the different growth forms and growth rates (measure by biomass) of *Lobaria oregana, Pseudocyphellaria rainierensis, Evernia prunastri, Usnea longissima* and *L. pulmonaria* in relation to thallus water content. In addition, thallus water content is related to growth (Gauslaa et al., 2006). There was a concern raised in this study though that changes in humidity could have interfered with measurement of thallus weight.

Soredia transplantation. Soredia consist of both algal and fungal partners. The algae are surrounded by fungal hyphae. They could grow readily in suitable environment. Numerous soredia are generally produced on thalloid surface. These diaspores could therefore be used both in culture and in transplantation. They are very small and could be attached to substrates. However, it has been found that some substrates have influence on growth of vegetative diaspores (Armstrong, 1988). Therefore, choices of materials for soredia transplantation have to be carefully selected. For example, Schuster et al. (1985) cultured soredia of some selected species of lichens on specimen-holders of scanning electron microscope (SEM) in natural areas and monitor the miniscule changes in their growth with the SEM. In 12 months, the soredia of *Hypogymnia physodes* produced hyphae of mycrobiont which were at arachnoidal stages on media and also produced small dichotomous lobes. Soredia of *Physcia tenella* occurred combination on media surface and also produced rhizines at the lower cortex. *Usnea filipendula* and *Parmelia sulcata* in this experiment could grow for 12 months developing into small lobes,

sizes of which *Xanthoria parietina* took 24 months to develop. In addition, unfavorable microclimate and pollutions could reduce soredia growth and development. Especially, unfavorable water-related conditions (*e.g.* continuously high humidity) can damage the thallus and soredia of *H. physodes*. Stocker-Wörgötter and Türk (1988) cultivated soredia of *Peltigera didacty* in a culture chamber under laboratory conditions. At the beginning of the cultivation, the humidity was nearly 100% from water condensation in the dishes. There was a continuous loss of water until the cultures had dried out. The relative humidity in the culture chamber varied from 50 to 60%. The cultures were kept in the culture chamber at 19 ± 2°C with an alternating daily cycle of 14 hours light and 10 hours darkness and a light intensity of 60-90 $\mu mol\ m^{-2} s^{-1}$ from fluorescent lamps. These results revealed that the soredia have no change during the first 2-4 weeks. However, within the first month, soredia started to germinate, adhering to the substrate and having similar spider web structure. Similar experiment was conducted by Schuster et al. (1985). They cultivated soredia of *P. didactyla* in the natural environment. Four months after cultivation, the soredia developed a cortex structure around lobe edges, forming foliose shapes. In 5 months, algae layer appears, being covered with fungal hyphae of cortical layer and developing as juvenile thalli.

In 1989, Stocker-Wörgötter and Türk transplanted soredia by scraping them on sterile soil in flowerpots similar to those used in the experiments of Webster and Brown (1997). They used camera microscope to determine diaspore growth. In 3 weeks, more than 70% of soredia had germinated. Cortex layer appeared in 10 months and primordia with a dentate margin

appeared after 3 months. After the fourth month, the indented marginal zones merged to a larger, smoother, flexible lobe margins. After the 5 months, the life cycle of *Peltigera didactyla* for vegetative reproduction was terminated. This experiment successfully cultured lichens for reproductive dispersal. Armstrong (1990a) transplanted soredia of *H. physodes* on barks to compare dispersal in differently ecosystems. He found that dispersal, growth and survival of soredia depended more on tree barks than tree locations in forests. Many soredia were transported by raindrops into new microhabitats on host trees on which they could adhere to rough barks rather than smooth bark (Armstrong, 1988, pp. 3-4). Consequently, characteristics of tree barks, on which lichens could grow, were studied and found that they had influence on lichen survival in several ecosystems (Moning et al., 2009).

Scheidegger (1995) studied transplantation of vegetative diaspores of *Lobaria pulmonaria.* The soredia were adhered on 8 mm wide pieces of bandages and transplanted on barks and moss covered surface of *Fraxinus excelsior* nearly original lichens. Transplanted samples were monitored under low temperature scanning electron microscopy (LSEM). In the first two months, 60% of transplanted diaspores were lost, being washed by rain or grazed by insects and invertebrates (Gauslaa et al., 2006a; Gilbert, 1991; 2002). However, the majority of the diaspores developed numerous confluent anchoring hyphae, which formed broad, fan-shaped contact zones to the substratum in 4 months. In 6 months, the apical and/or marginal growth zones developed into distinct forms. After 15 months, they formed lobules which were 0.3-0.5 mm broad and about 0.5 mm long. In 30 months, the developed

thalli were about 1 mm long. This experiment indicated that gauze could be suitably used as a substratum for lichen diaspora transplatation.

Later, Zoller et al. (2000) conducted an experiment similar to Scheidegger (1995). They transplanted both diaspores (isidia and soredia) of *Sticta fuliginosa*, *Leptogium saturninum* and *Menegazzia terebrata* on small plates of bandage which were adhered on tree bark. The diaspores were investigated by LSEM. In 2 and 16 months, 50% and 29% of the diaspores of *S. fuliginosa* and 46% and 19% of *L. saturninum* still remained on the gauze discs, respectively. Lobes resembling adult thalli were observed after 8 to 12 months in *S. fuliginosa* and *L. saturninum*. The same process took 16 months in the case of *M. terebrata*. All three species usually developed more than one thallus primordium (pseudomeristematic growth zone) per isidium or soredial cluster. The results showed that the juvenile development of the investigated species was not restricted by microclimate factors. The study had provided a useful transplantation technique to increase the number of lichen populations without damaging natural thalli. This work could potentially be used for *in situ* conservation of endangered lichen species.

In a more recent study of diaspora transplantation, Ott et al. (2004) studied soredia development of *Usnea antarctica* by installing small rock plates (1 cm^2) on the sample bases of SEM in natural sites. This work was similar to Ott and Jahns (2002). In one year, many soredia detached from substrates, presumably by wind, rain and snow. The soredia slowly grew during the first year, increasing in sizes and forming compact basal tissue and densely packed surface. In 3 year, juvenile structures had formed. In 5-6

years, the samples fully developed into adult thalli. The researchers suggested that development of juvenile thalli is controlled by a combination of ecophysiological and morphological adaptations. The growth patterns of this species were also observed in the studies of Schuster et al. (1985) in *Usnea* species.

Transplantation techniques were also used in studies on the capability of soredia distribution. Hilmo and Såstad (2001) studied the distribution of lichens from old forest into young forest and old pine forest by sprinkling the soredia of *Lobaria scrobiculata* on 240 controlled-sized branches of *Picea abies*. After 4 years, the researchers concluded that the conditions in the old growth forest were not suitable for the development and growth of soredia while those in the young forest promoted their growth and development. Sillett, McCune, Peck, Rambo, and Ruchty (2000) also found that the substrates and microclimate present in old forests did not favor the growth of *L. oregano*.

Isidia transplantation. Isidia also consisted of algal and fungal partners. They are different from soredia in that they have cortex layer to protected themselves. Isidia are bigger than soredia and thus are transplanted on various artificial substrates more easily (Ott & Jahns, 2002). Kershaw and Millbank (1970) studied isidia development of *Platismatia glauca* under control condition. They added N (0.2 g/l KNO_3) every 3 or 4 day. They found that isidia could be germinated/grown into 2 mm^2 to 12 mm^2 in 7 months. Hilmo and Såstad (2001) transplanted the isidia of *P. glauca* on spruce branches in natural habitat. They found that 34.6% of the transplanted isidia

grew to small lobes while others fell off during the period of transplantation owing to unsuitable microclimate, especially low light intensity which put a limit to the lichen's photosynthesis (Hilmo & Ott, 2002). Kon and Kashiwadani (2005) transplanted isidia of *Parmotrema tinctorum* on tree host *Cryptomeria japonica* in areas close to the where the lichen grew in nature using 5x5 cm^2 nylon bags. In 3 months, the transplanted isidia produced hyphae to adhere to substrates. In 6 months, the isidia produced several verrucae or protuberances 20-30 μm broad and 50-60 μm long. In 20 months, the protuberances developed into pale gray dorsiventral lobules 0.2-0.35 mm broad and 0.2-0.3 mm long. This study was seem to be that the growth of *P. tinctorum* more than *Platismatia glauca* and *P. norvegica* were studied by Hilmo and Ott (2002) after transplanted 21 months.

Influence of Microclimate on Lichens Transplanted

Microclimate and Season

Light intensity (PPFD). Photosynthetic process of lichens and higher plants alike is activated by light. Therefore, suitable illumination in the natural habitats is important for the growth of vegetative propagules. Different lichen species have different light saturation levels which influence their success in different natural habitat. For example, Coxson and Stevenson (2007) found that the high illumination at forest edge and low illumination at forest central areas affect the sizes of *Lobaria pulmonaria* thalli growing within the two areas. Hilmo and Ott (2002) found that an increase in growth rates of transplanted *Platismatia glauca* thalli in young and old forests correlated with an increase in light intensity. The transplanted lichens in the young forest had higher growth rates comparing to those in the old growth forest because of the higher light intensity in the former. However, it was found that exposure for a long period of time (24 hours) to 250-1000 $\mu mol\, m^{-2} s^{-1}$ light intensity would induce photoinhibition in lichens. Also, high light intensity also increased thallus temperature which led to rapid loss of thallus water content (Green et al., 2008).

While light intensity of 20 -1100 $\mu mol\, m^{-2} s^{-1}$ can induce photosynthesis in most lichens, different species can endure different levels of light intensity and have various points of light saturation (Green, Büdel, Meyer, Zellner, & Lange, 1997). For example, *Coccocarpia palmicola Pseudocyphellaria lividofusca* and *Usnea rubicunda* have light saturation points of 82, 146 and

549 $\mu mol\, m^{-2} s^{-1}$ respectively. Chlorolichens (lichens which have green algae as photobionts), such as *Parmotrema sp. Ramalina sp.*, could grow in high light intensity (Ahmadjian, 1993). Cyanolichens (lichens which have cyanobacterial photobionts), such as *Psuedochyphellaria* sp. and *sticta* sp., grow under low light intensity between 10-50 $\mu mol\, m^{-2} s^{-1}$(Campbell et al., 1998; Green et al., 2008). Therefore, tree heights and forest structures, which determine levels of light penetration through canopy, also affect the distribution patterns of lichen species (see Figure 1). The lichens in top canopy have higher light saturation than lichens growing at forest base. In addition, their growth rates increased with an increase in light intensity (Sillett & Antoine, 2004).

Temperature. The temperature (^{o}C) affects metabolic process in the way that it induces respiration which subsequently results in a decrease in chlorophyll contents (Sillett & Antoine, 2004). Lichens could grow in a wide temperature range from -15 to 55 ^{o}C. Some species could tolerate high temperature at 90-100 ^{o}C in a short period of about 30 minutes under sunlight in desert areas. Sancho and Kappen (1989) found that the lichen *Umbilicariaceae* which grew in alpine and montane belt areas and in the Arctic had maximal photosynthesis at 0-10^{o}C and 20^{o}C respectively. However, the average temperature in the temperate zone was only 2^{o}C. This results in the slow growth of temperate lichens. The low temperatures were found to inhibit lichen metabolic pathways and limited nitrogen fixation of both cyanolichens and chlorolichens (Sillett & Antoine, 2004). On the contrary, higher temperatures at 5-20^{o}C were found to promote photosynthetic

process of many lichens, especially crustose lichens in the tropic which had high growth rates when the average temperature was around 25°C (Green et al., 2008, pp. 165-166). It was also found that tropical lichens had higher growth rates than temperate and arctic lichens (Nimitr Osathanon, 2002). Lichen distributions of different boreal forests were also limited by variation in temperatures (Campbell & Coxson, 2001). Interestingly, canopy and ground temperatures were found to be much different and could contribute to lichen vertical distribution (Madigosky, 2004).

Relative Humidity. The water content (WC) in lichen thallus is directly related to relative humidity (RH). The water content importantly induced lichen photosynthesis and respiration, particularly when RH is saturated in the morning (Ahmadjian, 1993; Lange, Pfanz, Kilian, & Meyer, 1990; MacCune et al., 1996; Nash, 1996). The photoperiod of lichen photosynthesis is important for lichen growth in tropical forest. Madigosky (2004) found that the moisture at tree base of tropical forest was higher than that at the canopy during 11:00 am to 15:00 pm (5 to 20%). The difference in moisture depended on forest types and canopy structures. However, the lichen photosynthetic rates were highly related to thallus water contents, which are differed within various species. For example, chlorolichens could contain as much as 250 to 400% dry weight water in their thallus while cyanolichens could contain larger contents of thallus water up to 600 to 2000% dry weight (Nash, 1996; 2008a).

Lichens could endure the condition when thallus water content was as low as 5% dry weight in desiccation for many months without changes in

internal structures (Kappen, 1974). Moreover, dry thalli could resist harsh conditions like -196°C in liquid nitrogen, high air temperature at 60°C and high ultraviolet exposure (Beckett, Kranner, & Minibayeva, 2008). Chlorolichens are thought to have higher resistance in extreme habitats than cyanolichens and bryophytes (Sillett & Antoine, 2004).

Lange, Kilian, and Ziegler (1986) who studied water holding capacity and lichens photosynthesis found that chlorolichens and cyanolichens used different forms of water. Chlorolichens used water from fog while only water droplets could activate highest photosynthetic reactions in cyanolichens. However, lichens are able to absorb water from unsaturated air humidity to activate these pathway (Lange et al., 1986; Nash, 1996). Lange et al. (1990) observed no net photosynthesis (NP) when the relative humidity was lower than 85.5 and 86% in *Ramalina maciformis* and *Letharia vulpine,* respectively. Green et al. (2008) suggested association between water relation and photosynthesis of lichens; 1) photosynthesis was inactive during lichen drying. When thallus water contents were 5 to 10% or at 50% RH, metabolic pathways started and enzymes become active. 2) Thallus water content at 20% induced photosynthesis in green algae while cyanolichens needed 85 to 100% thallus water content. Lichens reached moisture compensation point (balance of photosynthesis and respiration) at approximately 22% water content. 3) NP increased rapidly to the maximum when WC was 70–150% (c. 80%) for chlorolichens and 300–600% for cyanolichens. 4) Maximum NP which corresponded to maximum WC were species specific (Lange, Büdel, Meyer, & Kilian, 1993). Some species maintained nearly maximal NP to the highest

water contents while another species had various degrees of depression (suprasaturation). The depressed NP is normally a result of increased diffusion resistances at high water content and thus a decrease in CO_2 diffusion.

Season. The growth rates of lichens in tropical forest were studied at Khao Yai National Park by Nimitr Osatharnon (2002). The maximum growth rate (1.5 mm/yr in rainy season, 2.4 in cool season and 3.6 in sunny season) was observed in *Parmotrema tinctorum*. Muir et al. (1997) found that *Lobaria pulmonaria* transplanted in the temperate could grow better in wet seasons (November - June) and had no growth in springs. In addition, initial sizes of transplanted lichens were not correlated with an increase in growth rates. Gauslaa et al. (2009) found that an increase in thallus area was correlated with dry mater gained. Caldiz (2004) studied lichens transplanted in Puerto Blest of Nahuel Huapi National Park at Argentina and found that the highest growth rates of transplanted lichens were observed during the cool seasons because of the low temperature and extra moisture from snow which extended lichens photosynthetic period. Furthermore, environmental changes in forest were much affected by season changes which these is avoidably influenced onto lichens due to that received high light intensity increasing in the autumn (Armstrong, 1993; 2002; Gauslaa & McEvoy, 2005).

Ecosystems and Substrates

Influent of ecosystems. Each ecosystem has specific climate and flora which limit the diversity and growth of lichens (Seaward, 2008).

Ramkamheang University, Faculty of Science, Department of Biology, Lichen Research Unit (2004) found many endemic lichens species specific to each type of forests at Khao Yai National Park. This indicated that the diversity of lichens in different ecosystems is affected by different forest climate conditions such as tall trees in the forest (Antonie & McCune, 2004; Seaward, 2008) and density canopy cover (Coxson & Stevenson, 2007). For example, tropical foliose lichens in secondary forest had highest growth rates (Nimitr Osatharnon, 2002). The growth rates of *L. pulmonaria* of which are studied by transplanted in old-growth forest in boreal forest types. While Sillett and McCune (1998) *Lobaria oregana* and *Pseudocyphellaria rainierensis* were observed survival in Douglas-fir forests in four ecosystems; old growth, mature, young and clear-cut forest were observed. The highest average annual and potential annual growth was observed in mature forest, while the highest mortality was found in clear-cut forest (Sillett, 1994).

Influent of substrates. Many lichens could be grown on several substrate types in the natural habitats (e.g. rock, soil, bark, splat and leaves). Moreover, some lichens could be grown on artificial materials; rubber, plastic, metal and glass (Seaward, 2008, pp. 274-278). Many study reported to influenced by component surface on lichens growth such as pH bark, element/mineral and surface types (Kershaw, 1985; Sloof & Wolterbeek, 1993), which has critical to the survival and adhesion of lichens reproduction. For example, the texture property of substrates has been affected to establishment of spores and diaspores germination (soredia and isidia); therefore, they could be well adhered on rough surface (e.g. bark, rock and

soil), where were also grown into juvenile thalli under suitable climate rapidly (Armstrong, 1992; Brodo, 1974; Nash, 2008a, pp. 1-8). Furthermore, some lichens species could be grown on bryophyte as they are assisted to prolong thallus water content of lichens (Sillett & Antoine, 2004). In the same way, Jüriado, lira, and Paal (2009) demonstrated to alkaline-acid of bark (pH bark), which could be changed by trees height, are affected on lichens grow. The pH bark increase associated to decrease of lichen abundance. In addition, Jüriado, lira, Paal, and Suija (2008) suggested that the substrates, which were covered by bryophyte, could be promoted growth and increased abundance of lichens flora.

Conservation

There have been focuses on methodologies for lichen transplantation for conversation of rare or endemic species and also for growing new species in destructed forests (Scheidegger et al., 1995; Scheidegger & Werth, 2009). These processes would be developed for transplanted lichens in tropical forest to conserve lichens in the environment (Gaio-Oliveira, Dahlman, Maguas & Palmqvist, 2004; Gauslaa et al., 2006a). In the future, climate change and changes in ecosystems are deemed to affect lichen population in the wild, potentially decrease their availability and diversity (Moning et al., 2009; Scheidegger & Werth, 2009). Currently, there are more studies on lichens on the aspects of the secondary compounds they produce, which could potentially be used in medicine and pharmacological industry. These lichen

compounds had been used to make perfume and medicine (Elix & Stocker-Wörgötter, 2008, pp. 130-133). Some lichen species were used in food industrial while many were used in studies of air pollution (Garty, Weissman, Cohen, Karnieli, & Orlovsky, 2001; Kansri Boonpragob, 1993; Pimwong Subsri, 2002). In the present, lichens are being threatened by air pollution, habitats loss and a climate change (Aptroot, 2009; ven Heark, Aptroot, & van Dobben, 2002). Therefore, the studies for methodologies of lichen transplantation in tropical forest would greatly benefit conservational work on lichens biodiversity and forest management.