CHAPTER 5

DISCUSSIONS, CONCLUSIONS AND RECOMMENDATIONS

Discussions

Survival of Thallus Fragments of Site-Specific Species Transplanted to Other Ecosystems

Survival of Cool Inhabited Species in Warmer Climate

Thallus fragment of the four species, *H. kingii*, *H. osseoalba*, *H. lepidota* and *P. chozoubae*, naturally grow in the cool-wet habitat of the LMF hardly adapted in the warmer areas at TRF, DEF and SF after transplantation. Proportion of survived thalli in these ecosystems was less than 9% after 43 months of transplantation. The highest proportion of survived thalli was located in SF, and subsequently lower in DEF and TRF.

Among the four species from LMF, *H. kingii* showed the best adaptive capacity in all warm transplanted sites (see Figure 29). Although this species survived and grew better in SF where illumination was intense, however, it was able to survive in TRF and DEF, where light was limited as well. It indicated that *H. kingii* could grow in wide ranges of light intensity, and temperature. This species should have better chance to survive then the other three species under changing climate. Present restrict distribution of *H. kingii* only in LMF suggested that this species may be vulnerable to other factors, which remain unknown. Possibly this lichen may not be able to compete with other species, or information on its distribution is insufficient. This information requires further studies.

H. kingii normally inhabited canopy and open areas of LMF (Kawinnat Noicharoen, 2002). This evidence suggests that *H. kingii* could tolerate warmer temperature under sufficient illumination for photosynthetic carbon assimilation to produce organic matter for supporting growth.

H. osseoalba could survive better than other two species, *P. chozoubae* and *H. lepidota*, in TRF and DEF. It indicated that *H. osseoalba* was able to adapt to low light and warm temperature in these two forests. This species normally inhabits at mid-trunk to canopy and open areas in LMF (Kawinnat Noicharoen, 2002), where illuminations from the sun are adequate for carbon assimilation.

Despite survival of *P. chozoubae* and *H. lepidota* in SF, but they almost completely disintegrated in TRF and DEF. It suggested that these two species could not adapt to low light intensity, but may be able to adapt to warmer temperature. *H. lepidota* and *P. chozoubae* seems to be the most sensitive ones to warm climate. *H. lepidota* was reported to specifically found in cool areas of the high montane forests and other ecosystems at 1200 to 1500 m.a.s.l. in Thailand (Ramkamheang University, Faculty of Science, Department of Biology, Lichen Research Unit, 2004). It's distribution at 2000 to 3000 m.a.s.l. is reported by Swinscow and Krog (1976). Likewise, *P. chozoubae* is a species grow specifically only in LMF in Thailand. It implied that these two species had less capacity to adapt to changes, especially illumination, and could extinct from their present habitats under climate change.

High survival in Secondary forest (SF). SF in this study was located in the previously deforested TRF, where rainfall was high and illumination was intense because plant communities were during stages of succession. The trees were not fully developed into the mature forest, therefore canopy was relatively open. The tree trunks, where lichens were transplanted had the highest illumination (107 µmol $m^{-2}s^{-1}$) among the three ecosystems. Consequently, humidity was not as high as TRF because high temperature, as a result of intense illumination, drove evaporation process. Despite warmer temperature (22 °C) and dryer atmospheric humidity than the other sites, SF supported the largest proportion of survived thalli of the transplanted materials. It underpinned the overriding importance of light as a key factor that determined survival of lichens in the tropical ecosystems, where vegetation severely competes for light energy.

Almost a quarter of the transplanted materials of *H. kingii* survived in this location. It indicated that this species adapted well in sun lit habitat. In addition, compatible habitat factors that species grew before and after transplantation affected successful establishment in new location.

Tropical rain forests (TRF). Most of the transplanted species from LMF could not establish in TRF. This forest had the lowest number of the survived thalli after transplantation. Despite this site had average temperature and relative humidity recorded 21.6 °C and 80%, which are optimum for metabolic processes of lichen (Green et al., 2008), nevertheless illumination was low (<43 μmol $m^{-2}s^{-1}$). This condition limits distribution of lichen and other photosynthetic plants due to insufficient light as a source of energy (Green & Lange, 1991; Green et al., 1997; Green et al., 2008; Palmqvist et al., 2008).

Lichens require light intensity of about 82-766 μmol $m^{-2}s^{-1}$ (record from temperate by Green et al., 1997) or up to 300 μmol $m^{-2}s^{-1}$ in early morning during photosynthetic active period (Lange, Reichenberger, & Walz, 1997). TRF in this study is a mature forest with close canopy. Illumination during 6:00-10:00 a.m. was only 43 to 129 μmol $m^{-2}s^{-1}$ (see Table 3) which was not enough for carbon assimilation of the transplanted lichens. Therefore, structure of forest was among the factors that determine lichen survival. Moning et al. (2009) reported that lichen diversity was affected by stand structure than climate in temperate montane forest. Apparently, the warm-wet TRF composed of taller tree than other ecosystems, light penetration through the canopy was reduced.

Dry evergreen forest (DEF). Survival of all transplanted species in DEF was slightly more than those in TRF. Nevertheless, only small proportion of *H. kingii* and *H. osseoalba* survived. Forest structure and consequently microclimate influenced establishment and growth of the transplanted lichens. During early morning in DEF atmospheric humidity was relatively dry and illumination was low (29-66 μmol $m^{-2}s^{-1}$), whilst thalli had been moistened by atmospheric humidity through out the night. This situation was critical for photosynthesis carbon assimilation, and hence limited lichen distribution into this forest. Surprisingly, the highest average growth rate of *H.*

lepidota was measured in this area. The factors that involved with this achievement were unclear.

Survival of Lichens in Cool Area Transplanted from Warm Areas

After transplantation to cooler habitat at LMF, five species of lichens composed of *P. argyracea*, *R. abstrusa* and *R. subconnivens P. rubromarginatum* and *D. picta* from warm locations had higher proportion of survived thalli comparing with those transplanted from the cool to the warm sites. About 30-70% of the transplanted thalli of these species survived and grew. This evidence suggested that lichens from warm areas in the tropic could adapt better in the cooler climate, contrary to those from the cool to warm habitats. However the ability to adapt varied among species.

The cool tropic in this study was represented by LMF, where canopy was partially open. Consequently, sub canopy and tree trunks were well illuminated. Average temperature was about 20 °C, which was lower than the warm sites, and more humid than the others. This condition had profound effects on photosynthesis and organic matter production of the lichens. The consequent influences on survival and growth of lichen will be discussed later.

In general, optimum temperature for the maximum photosynthesis is 20-25 °C. Lower or higher temperature than this level slows down photosynthesis rate and consequently lesser production of organic matter. By contrast, respiration increases along with temperature raise, and as a result organic matter is used up causing growth decline and death (Green et al., 1991; Green et al., 2008; Palmqvist et al., 2008).

R. abstrusa was the most successful species in the new cool location with 67% of thalli survived. This species inhabited at mid-trunk to canopy at TRF and SF (Ramkamheang University, Faculty of Science, Department of Biology, Lichen Research Unit, 2004). This species probably had the best adaptive capacity to changes in temperature and humidity as long as that light was not limited.

P. argyracea seems to be able to survive in the cool area quite well. This species, which was found in deep shad of moist habitats in TRF, could adapt to high humidity and cooler areas of LMF relatively well.

P. rubromarginatum is a species that was described as a new species collected from SF at KYNP (Titiporn Pooprang, Kansri Boonpragob, & Elix, 1999). The distribution is relatively limited.

D. picta is a common lichen in Thailand. It is found in almost every forests, rural and urban areas as well as islands in Thailand (Sittiporn Parnmen & Kansri Boonpragob, 2003; Wasana Cheusook et al., 2005). This species inhabits SF and dry dipterocarp forest at KYNP, without any record from LMF. However, it had low number of survivors in the cool transplanted area, which mean that this species may prefer warm areas over the cool one.

The lowest number of survivors in the cool site was recorded from *R. subconnivens*. This species occupied canopy at TRF, and rather specific to this forest. It has no report from other locality (Ramkamheang University, Faculty of Science, Department of Biology, Lichen Research Unit, 2004). *R.*

subconnivens displayed low adaptive capacity in low temperature of LMF although atmospheric humidity was high comparable to its original habitat at TRF. However, its capacity to adapt to warm temperature is unknown. This species seems to prefer warmer climate, and may flourish in the warming habitats.

Growth Rate of Site-Specific Species After Transplantation to New Locations

Temperature, relative humidity and light intensity are important factors for lichens survival and growth in each forest (Moring et al., 2005; Sillett & Antoine, 2004). Growth rate was one of the factors used to examine the capacity of lichens adapted to new habitat. This study revealed that average growth rate of the five lichens transplanted to the cool area was higher than lichens transplanted to the warm areas, given thallus sizes at the start were comparable. Greater growth rate in the cool habitat was possible due to high photosynthetic and low respiration rates of the lichens in the LMF. This forest had average temperature of about 20 ^{o}C, which is optimum for photosynthetic process, whereas the TRF, DEF and SF had averages of 21.6, 22 and 23 ^{o}C respectively. Temperature beyond 25 ^{o}C tends to reduce net photosynthesis whilst enhance respiration (Green et al., 2008, pp.165-166; Insarov & Schroeter, 2002; Lange, Budel, Meyer, Zellner, & Zotz, 2004). This condition caused reduction in growth. Moreover, low growth and disintegration of the transplanted thalli in the warm habitats might have been caused by effects of temperature on these two vital processes. However, lower growth rates of *H.*

lepidota and *P. chozoubae* were associated with moisture more than temperature.

Therefore, it is probable that lichens from the warmer habitats may migrate to the cool area and compete with the original inhabitants under changing climate.

The transplanted *D. picta* in LMF grew 1.7 mm/yr, which was lower than 7.2 mm/yr reported from its natural habitats in SF, DDF (Nimitr Osathanon, 2002). The differences might cause by stages of growth phase and climate. The transplanted thalli were obviously fall in the establishment phase, which growth rate is low. By contrast, the reported growth rate was measured from various sizes of thalli including exponential phase, which has the highest rate of growth during development.

In fact, *D. picta* have been found in LMF of other areas in Thailand, but they absent in LMF at KYNP (Ramkamheang University, Faculty of Science, Department of Biology, Lichen Research Unit, 2004) Although this areas was favorable for germination of diaspores including those of the warm species. However, habitat competition with other plants was high. This factor limits establishment of juvenile thalli. Scheidegger (1995) suggested that moist and cool habitats enhance competition by other epiphytes and bryophytes (Seaward, 2008).

Other Influencing Factors

Grazing by insect and invertebrate infestation led to disintegration of the transplanted thalli. The transplanted *P. rubromaginatum* and *D. picta* were threatened by these factors in the LMF. Gauslaa et al. (2006a) reported that snail feed on transplanted thalli of *Lobaria pulmonaria* in the temperate forest. Asplund, Solhaug, and Gauslaa (2008) reported that lichen-feeding mollusks could strongly limit the survival of young lobules of *L. pulmonaria* (Vatne, Solhøy, Asplund, & Gauslaa, 2010).

Survival and Growth of Vegetative Propagules on Host Trees in Various Ecosystem

Under changing world caused by human development, lichens which are most sensitive to environmental change, especially climate change and atmospheric pollution are threaten to extinction (Scheidegger & Werth, 2009). Lichen is a component of ecosystem and is essential for existence and integrity of ecosystem, which is life supporting system. More importantly, lichens possess novel natural products which are specifically found only in lichens. Assisting lichens to survive in natural habitat through *in situ* conservation is a mean to protect and conserve genetic diversity of organisms for sustainable development. This study explores the possible impacts of habitat changes and appropriate methodology for conservation and enhancing production of lichens by transplantation of vegetative propagules.

Diaspores Germination, Survival and Growth

Vegetative diasporas, isidia and soredia, are produced in large quantity by many lichens as means for distribution (Seaward, 2008, pp 274-280). They contain both partners of the lichen, photobiont and mycobiont. Under suitable habitat they are ready to germinate and grow (Armstrong, 1988, pp. 3-4; Frahm, 2003; Seaward, 2008, pp. 274-280). This mean of distribution is advantage over thallus fragment because it requires less lichen material. It is even much more advantage over propagation by discharging spores, from mycobiont, which probably needs a long time to find proper photobiont for symbiosis (Ahmadjian, 1993). Assisting propagation of lichens by transplantation of isidia and soredia to different natural ecosystems on four aspects orientated to the sun at three levels of tree trunks found that germination and survival of the two vegetative propagules were slightly different among these microhabitats. Armstrong, (1988; 1990a), Buldakov (2010) and Palmqvist et al. (2008, pp. 182-215) reported that microclimatic factor is very important for diaspores germination.

During early stage of transplantation, 12 months, both isidia from *P. tinctorum* and soredia from *P. praesorediosum* had the most successful germination in LMF at mid trunk level. Nevertheless, the isidia had larger proportion of survival on the East aspect of tree trunk, whereas those of the soredia developed well only on the North aspect. In addition, overall proportion of survival of the isidia was greater than the soredia in this ecosystem, accounting for 63% and 51% of the original transplanted materials.

After 25 months after transplantation, the newly emerged juvenile thalli from the isidia and the soredia were grown over by the common algae in the tropic, *Trentepohlia* sp. This algae is easily recognized by orange color, containing large amount of carotenoid pigment which helps protection from sun damages. *Trentepohlia* grows abundantly in the tropic in well illuminated area. It can be found as free living and as photobiont in lichens (Friedl & Büdel, 2008). This evidence suggested that tropical cool climate, well illuminated and high humidity favor establishment, germination of isidia and soredia of lichens, but also enhanced growth of other natural flora, the *Trentepohlia* as well. In this study lichens which are slow growing flora lost spatial competition to the fast growing algae *Trentepohlia*. They were completely disintegrated from the transplanted sites at LMF at the end, 64 months, of investigation.

By contrast, TRF and DEF did not suitable for propagation of these diasporas as germination and survival in these ecosystems were quite low, throughout the investigations. This was probably due to low illumination in TRF and DEF. It suggested that high atmospheric humidity in TRF was not benefit to lichen as long as light energy was insufficient for carbon assimilation. In addition, warmer temperature in these forests might enhance respiration process, which could not be compensated by low photosynthetic rate under low light intensity (Green & Lange, 1991; Green et al., 2008, pp 165-167; Palmqvist et al., 2008, pp. 199-206).

Interestingly, long term investigation of 64 months, indicated that SF could support survival of the isidia and soredia better than the other ecosystems, despite germination was lower than LMF at early stage of transplantation. Secondary forest had drier atmospheric moisture, and warmer temperature due to open canopy that allow sun light to illuminated the growing habitats of lichens. The diurnal cycles of wet and dry, light and dark probably fitted for symbiosis and development of photobiont and mycobiont of lichen. In general, successful symbiosis and development of lichen require such cycles, which is relatively specific for each species (Nash, 2008a, pp.1-3; Honegger, 2009). This investigation suggested that diurnal cycle of light and moisture in SF was appropriated for long-term development of lichen thalli, and likely inhibited competition from the *Trentepohlia* and other algaes. This condition probably caused by moisture limited rather than photodamage because the *Trentepohlia* algae possess large quantity of carotenoid pigment that help protection from excess energy of the sun.

Therefore, LMF was the best place to start propagating lichen from isidia and soredia by fixing at the middle of tree trunk on the East and South aspects for the isidia, and only the East aspect for soredia. At these microhabitats, cool, moist and well illuminated, isidia germinated into juvenile lobes of lichens as much as 80%. Soon after 25 months, the juvenile thalli should be transferred to SF for achieving the greatest number of live thalli.

Despite present microhabitat at LMF that provided the best nursery ground for development of vegetative diaspores of lichens. However, this type of habitat occurred on elevated mountain in the tropic, and has high potential to be threaten by global warming (Aptroot, 2009, pp. 401-408; Intergovernmental Panel on Climate Change, 2001). Impacts, vulnerability and adaptation of flora, including lichens, and fauna as well as resilience of this ecosystem need long term investigation. Nevertheless, this study underlined the importance of climate as a trigger factor for establishment and existence of lichens. It could be used as indicator organism which relevant to other situation.

Survival of Thallus Fragments

Among the three vegetative propagules of lichen, thallus fragment had the fastest germination and development of into new thalli. This is because symbiosis between photobiont and mycobiont is more advance than those in isidia and soredia. Stratification of photobiont and mycobiont in, the progress stage of symbiosis development in lichen, already occur. Therefore, thallus fragments are ready to develop new lobes and thalli. Despite, thallus fragments is an efficient mean for propagating lichen, nevertheless, the disadvantage is depletion of lichen resource (Scheidegger and Werth, 2009). However, in some cases, propagation by thallus fragment is necessary because it is less sensitive to surrounding environment than isidia and soredia.

Survival of thallus fragments of *P. tinctorum* after transplantation varied greatly among ecosystems, levels and aspect orientation. Three microhabitats had much higher proportion of survivors than the others. These included LMF on canopy at the north facing aspect, SF at the tree base on the south aspect, and LMF at the middle of the trunk on the west. Aspect orientation in LMF did not always have profound effects on development of the thallus fragment. Proportion of survived thalli was only slightly different among the four aspects. By contrast, aspects of the trunk in SF had significant effects on survival of thallus fragments. Proportion of survived thalli varied greatly among the four aspects, of which survivors on the south aspect was almost double of the other three sides.

Successful development of thallus fragments was largely relate to illumination (see Figure 68). At these microhabitats light intensity in early morning during metabolic active period of lichen was about 100-200 µmol $m^{-2}s^{-1}$, whereas humidity and temperature were 87% and 22- 19.2 °C.

Interestingly, mid trunk of the SF where illumination was as high as that of the LMF, nevertheless proportion of survived thalli was much lower. This evidence could possibly attributed to drier atmospheric humidity in SF. The mid trunk of SF where germination of isidia and soredia was the most successful, could not support development of the thallus fragments. It illustrated that development of the three vegetative propagules, isidia, soredia and thallus fragment need different microclimatic condition.

By contrast, DEF and TRF barely supported any development of the thallus fragments on neither levels nor aspects. These microhabitats had low illumination, except the canopy of TRF where light intensity was the highest among all microhabitats. Nevertheless, proportion of survivor was not as high as other locations with similar high light intensities. This was probably cause by intense illumination, low humidity, insect infestation and competition with crustose lichens (Antoine & McCune, 2004, Gauslaa et al., 2009, Armstrong & Welch, 2007).

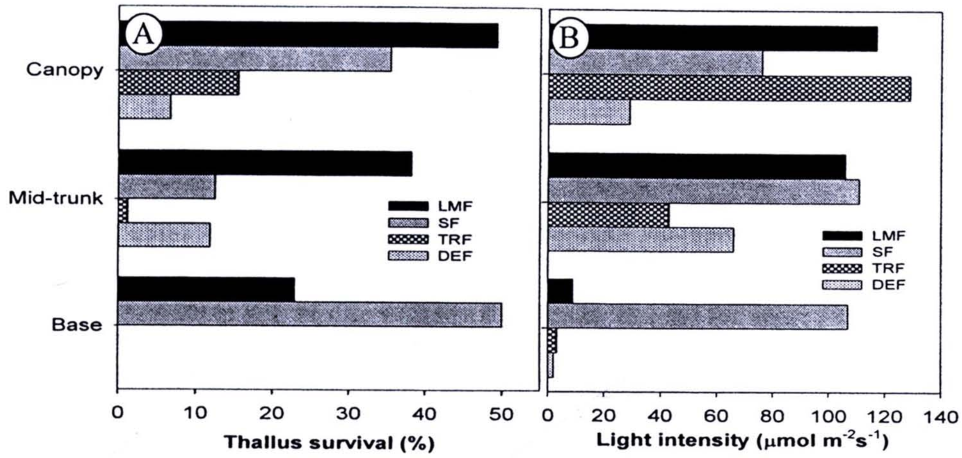

Figure 68 Comparing survival of thallus fragments of *P. tinctorum* with light intensity along tree trunks in various ecosystems after transplantation.

Note. Survivors at canopy, mid trunk and tree base in different forests (A) light intensity at different levels along tree trunk in different forests (B).

Growth of Thallus Fragments After Transplantation

Growth of thallus fragments of *P. tinctorum* along all three levels on the tree trunk at LMF had the highest rates comparing with those recorded from the same levels and aspects of others three forests. Although all four aspects of the canopy, mid trunk and tree base of this forest support high growth of the transplanted thallus fragments. Nevertheless, the eastern aspect orientation had the highest rate of growth. It probable related with illumination as high as 106-117 μmol $m^{-2}s^{-1}$ at the mid trunk and canopy levels of LMF during the metabolic active period of lichen in early morning (see Figure 69). Light intensity at these two levels could be considered as low illumination in general, however, it could be regarded as high light comparing with other levels in the other three forests measured in this study. Light saturation for photosynthesis of the dominant foliose species in tropical rain forests is approximately 500 μmol $m^{-2}s^{-1}$ (Green & Lange, 1994; Green et al., 2008, pp. 164-165; Zotz, Schultz, & Rottenberger, 2003). Illumination intensities measured at these two levels were about a quarter of the saturated level, which supplied certain amount of energy for photosynthesis and biomass production, and hence enhanced lichen growth. Zotz et al. (2003) reported that *Parmotrema endosulphureum* with *Trebouxia* sp. photobiont, achieves light compensation level at 35 μmol $m^{-2}s^{-1}$ and light saturation of net CO_2 uptake at about 330 μmol $m^{-2}s^{-1}$. The same genus, *P. tinctorum* used in this study, could have similar light requirement. Recently, Piccotto and Tretiach (2010) reported that *Parmotrema perlatum* had the light compensation point and saturation of net CO_2 uptake at 20 and 360 μmol $m^{-2}s^{-1}$ respectively. These levels are comparable with those measured at the mid trunk and canopy levels of LMF in this study.

Despite very low light intensity at the tree base in this forest (see Figure 69B), average growth rate of the transplanted thallus fragments were surprisingly high. This was probably due low light intensity at this level that prevented distribution of other flora, and consequently less spatial competition. Only lichens which adapted to low illumination could live and grow. The microhabitat at the tree base at LMF had period of high humidity, RH over 80%, longer than other levels at the same and other forests. This condition prolong photosynthetic period of the lichen, accordingly more organic matter was produced and growth was elevated.

Interestingly, growth rate of thallus fragments at SF was the second highest, next to LMF, as averaged from the three levels along tree trunks in SF. The south and the west aspects exhibited higher rates than other two sides. High growth rates at the canopy and base of the tree trunk caused by similar factors. The base of the tree where illumination was higher than the canopy, however , humidity was also high because this forest received high amount of rainfall. Evaporation from wet soil was the source of moisture, which probably prolong photosynthetic period in this microhabitat and enhanced growth of thallus fragments. Comparing with the canopy where lower illumination may slow down evaporation, but the intensity was considered high enough to achieve carbon gain from photosynthetic process. Light saturation for photosynthesis of *P. tinctorum* is about 350 µmol $m^{-2}s^{-1}$, while light compensation point was 48 µmol $m^{-2}s^{-1}$ (unpublished). Illumination beyond these levels is the excess energy which lichen is unable to use. The canopy and base of SF recorded illumination of 76 and 107 µmol $m^{-2}s^{-1}$, which was within the positive gain of carbon assimilation.

By contrast, the highest illumination recorded in this study was measured at the canopy of TRF. However, growth of the lichen thallus was not proportionally corresponded with increasing light intensity (see Figure 69). It seemed like high temperature and low humidity played major role at this level. High illumination follows by increasing temperature drove evaporation from substrate including lichens. Lichen thalli at this microhabitat probably dried faster than those in the cool humid condition, i.e. LMF. Therefore, photosynthesis period was shortened, and consequently led to low growth rate. Zotz et al. (2003) reported that CO_2 uptake of *P. endosulphureum* terminates around noon due to desiccation of thallus.

The DEF only supported development and growth of the thallus fragments at the canopy and the middle of the trunk, whereas tree base could not carry this lichen. The canopy of DEF had quite low illumination comparing with the middle of the trunk (see Figure 69B). Nevertheless, growth rate of the transplanted thalli averaged from the canopy was higher than the mid trunk. Average high growth rate of thallus at the canopy level was contributed by those measured from the eastern aspect of the trunk, where illumination was high (30 $\mu mol\ m^{-2}s^{-1}$). Other three aspects of the trunk had rates of growth about half of this value. The middle of the trunk where illumination was higher, might led to fast drying of thalli and shorter time for photosynthesis. No survival and growth of thallus at the lowest level of tree trunk, where illumination was almost negligible during early morning, demonstrated that light limited development and growth of lichens at this level (Antoine & McCune, 2004; Zotz & Schleicher, 2003).

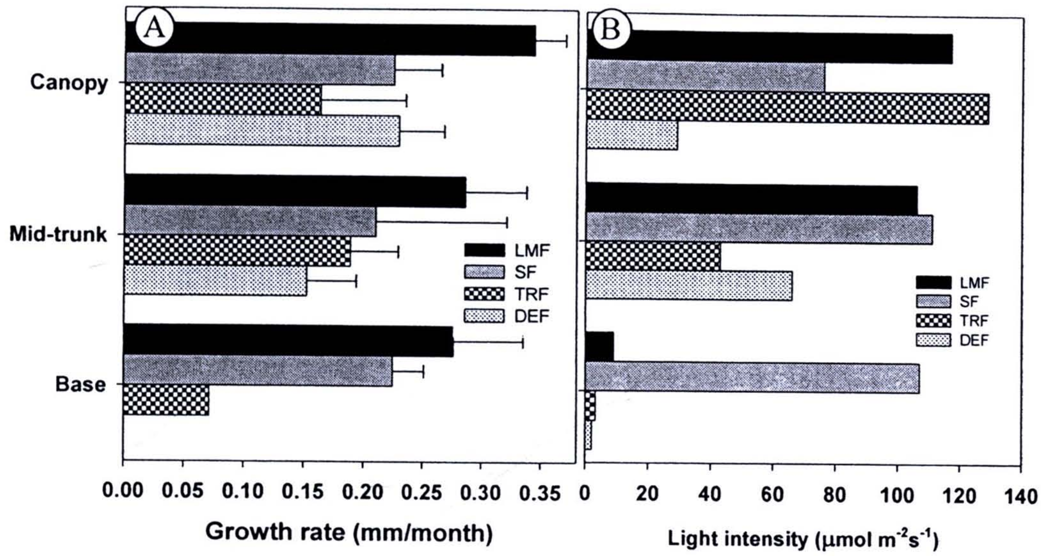

Figure 69 The average growth rate of thallus fragments of P. tinctorum on three levels along tree trunks after transplantation (A) light intensity at the three levels during metabolic active period (B).

Regeneration of Vegetative Propagules of Lichen on Artificial Substrates in Different Ecosystems

Lichens can be found on several kinds of substrates both natural, such as bark of tree and rock, and man made materials such as metal plate and concrete (Seaward, 2008, p. 278). Lichens only use substrates for attachment and grow; nevertheless, property of substrate is important for establishment and development of lichens (Armstrong, 1983, p 9; Nash, 2008a).

Regeneratuin of Vegetative Propagules of Lichens on Artificial Substrates in Different Forests

This study found that six artificial substrates including wood, ceramic tile, concrete block, glass bottle, rubber tile and galvanized iron in three different types of forest namely LMF, TRF and SF had different influences on regeneration of vegetative propagules of lichens after transplantation. At early stage of transplantation all vegetative propagules were able to regenerate in every condition, although at varying capacity. However, at the later stage, 35 months after transplantation, they barely existed. Glass bottle was the only substrates that the isidia, soredia and thallus fragments could grew and thalli remained until the final investigation period. TRF could support the survived thalli better than the other three forests.

Capacities of Vegetative Propagules to Regenerate

Thallus fragments had larger proportion of survived samples than isidia and soredia after a few months of transplantation in most locations. Nevertheless, the isidia existed in the highest quantity after long period of time, with soredia ranked the second. Thallus fragments used were at advance stage of development consisting of proper layers of photobiont and mycobiont in mutualistic relationship. Therefore, they were ready to germinate well before the isidia and soredia, which the two partners only formed loose association, without development into recognizable thallus. The isidia germinated in larger proportion than the soredia in all three transplanted sites indicated that the former vegetative propagule was able to adapt in the new environments better than the latter. It was possibly caused by different structure of the two vegetative propagules. The isidia has cortex, the compact layer of mycobiont hyphae bound over the surface, which help protection form unfavorable surrounding environment. Contrary, soredia lacks of this protection. It only composes of loosely interwoven of fungus mycelium surrounded the photobiont cells. It is sensitive to even small change of the environment (Buldakov, 2010).

The Influences of Artificial Substrate

The artificial substrates used for transplantation of vegetative propagules in this study had different physical and chemical properties, which affected regeneration and growth of lichens in a different way. At the early stage of transplantation, the three vegetative propagules seem to be able to germinate on almost all kind of the substrates in every forest, except on galvanized iron. The last substrate barely supported any development of the lichen propagules. Interestingly in a long run, only glass bottle could support growing thalli that germinate from the vegetative propagules in all forest, despite proportion of the survivors were varied.

Glass bottle. Smooth slippery surface of the glass bottle surprisingly supported germination and development of all vegetative propagules through long-term investigation. This information rejected our understanding that rough surface favors establishment and growth of lichens over the smooth ones as reported elsewhere (Schroeter & Sancho, 1996). Instead, it indicated that chemical property was probably more important than physical property of the substrate for progressive development of lichen vegetative propagules. Glass bottle surface is relatively neutral in term of acid-base property. In addition, smooth curve surface of the bottle seems to assist water flow, thus preventing over saturation of thallus. Under prolong wet condition and low light intensity, lichen respires heavily and consequently lost organic carbon, which lead to death of lichen (Seaward, 2008, pp. 274-280). The alternate wet and dry cycle over the glass bottle was likely to favor development of lichen. This condition was unsuitable for growth of other natural flora, and likely to minimize spatial competition of other organisms (Scheidegger, 1995; Zoller & Scheidegger, 2000)

Wooden board. This substrate should be considered as semi artificial because they were pieces of timber which were natural in origin. Only two thallus fragments were able to survive on wooden surface in LMF through the end of the investigation. Other vegetative propagules could not exist on this substrate in any forest in long term. This was probably caused by combination of factors, physical and chemical properties of substrate particularly used in this experiment. The wood board used might be recently sawed off from timber, which did not age long enough with the environment. The lichen *P. tinctorum* that commonly grows on old pieces of wood on fences and bridges was unable to grow on this substrate.

Galvanized iron. Despite metal signs, traffic signs at KYNP as well as abandon cars at other locations have lichens grow on them, however, the galvanized iron in this study could barely support any vegetative propagules. None of the soredia was able to germinate and survived on this substrate either short or long-term periods. At the very early stage of transplantation, only few thallus fragments germinated, and thereafter disintegrated rapidly. It was probably caused by toxicity form the metal. Eisler (1993) reported that the photosynthesis in lichens is inhibited when zinc (Zn) is more than 178 mg Zn/kg DW in soil. Nash (2008c, p. 245) reported that Zn is significantly reduces net photosynthesis at concentrate 300-500 ppm. The inhabitable metals for lichens are those exposing in the particular habitats for long period of time. They integrate and become homogeneity with environment, and toxicity was possibly washed away.

Ceramic tile and Concrete block. These two substrates had high pH values, which was probably the main cause of uninhabitable and grow of vegetative propagules in long period of time.

Rubber tile. This substrate bent after exposing in the transplanted sites, and may consist of materials that were not fit for lichen regeneration. It also accumulated heat load during the day, which could not support lichen regeneration.

The Influences of Ecosystems

During the course of development of the transplanted vegetative propagule of lichens, microclimatic condition in the four forests differently affected development and survival of lichens.

Lower montane forest. This forest provided the best nursery ground for germination and establishment of all vegetative propagules. The highest proportion of germination on all substrates was observed from this ecosystem after five months. It indicated that cool, moist and well illuminated habitat, of this forest, was prime important for starting development of lichen propagule. However, at the later stage only isidia and thallus fragments were able to survived in this forest and only on glass bottle. The soredia was completely wiped out from every kind of substrate in this forest. The young juvenile lobes were unable to develop further because algae and fungi invaded the substrates. Environmental condition of this forest also favored growth of other floras, particularly the *Trentepohlia* algae.

Secondary forest. This forest was the most suitable habitat for development and growth of vegetative propagules after germination. The largest proportion of newly germinated thalli remained in this forest after 10-40 months, although degenerated thereafter. It indicated that high illumination, relatively dry condition and higher temperature in this forest was appropriated for later stages of development of thalli. This microclimatic condition also limited natural enemy, the fast growing algae, due to its dryness. Unlike other floras, lichens have competitive advantage over the others organisms by their ability to utilize atmospheric moisture for living. Lichens absorb atmospheric humidity during the night, when relative humidity was over 80%, lichen thalli are moistened in early morning and photosynthesis resume when early morning light is obtained (Green & Lange, 1994, pp. 319-341; Green et al., 2008, pp. 152-181; Zotz et al., 2003). Another vital factor for thallus development is proper diurnal cycle of wet and dry condition, which may be fitted the requirement of the lichen used in this study.

Tropical rain forest. On a long-term basis, low illumination, high atmospheric humidity and relatively high temperature in this forest could support thallus development and survival from vegetative propagules of lichens better than other ecosystems. Under low illumination, and high atmospheric humidity lichens were able utilize these limited resources better than other flora because of its ability to used atmospheric water as mentioned earlier.

Dry evergreen forest. All the transplanted vegetative propagules could not established in this forest after 25 months of transplantation. The lichen substrates were grown over by climbing plants. Therefore this forest could not be used for propagation of lichens. Low illumination and dry habitat in this forest were unfavorable for lichen development.

Survival of Vegetative Propagules of Lichen After Transplantation at SF

Present SF at KYNP was previously TRF before settlement of villages. These villages were moved out after the area was declared as a national park. Thereafter succession process started, which the vegetations were not at mature stage of the original forest at present. It has open canopy, which allows sun light to illuminate through the ground. This kind of habitat, bright illumination, moist and moderate temperature, is appropriate for lichen growth. Therefore, thallus fragments of the lichen *P. tinctorum* were transplanted to ceramic tile, polycarbonate plate, and nylon net for mass production of lichens. These substrates were easily accessible and low cost. Large quantities of lichen biomass may be needed for uses in conservation and sustainable utilization i.e. natural products, monitoring air quality. Successful and failure of this experiment are discussed as follow.

Survival of Thallus Fragments on Polycarbonate Plates Under Canopy of SF

Polycarbonate plate had no capacity to hold water, and ventilation was poor. The thallus fragments had higher number survivors, but growth rate was lower than those transplanted to ceramic tiles. Thalli on polycarbonate plates were over saturated by water in rainy season, which led to blocking of CO_2 diffusion to carboxylation sites of the photobiont (Green & Lange, 1991; Green & Snelgar, 1981; 1982). In addition, they could be exposed to intense sun light while thallus was over saturated, causing accumulation of heat and led to thallus temperature increased. This condition affected carbon balance of lichen through rates of metabolic processes. In general, respiration increases with raising temperature, of which depleting organic carbon uses for growth. This might be a causing factor of low growth in rainy seasons (see Figure 70). Moreover, growth rates on this substrate had declining trend over a long-term. Khersow (1985) and Palmqvist et al. (2008, pp. 193-198) suggested that the photosynthetic process could be limited by high saturation of water in thalli. In addition, rhizenes of *P. tinctorum* attached loosely on surface of polycarbonate plates, which easily washed away in heavy rains and thunder storms.

Survival of Thallus Fragments on Plastic Nets and Ceramic Tiles Under Shading Net After Transplantation in SF

Plastic nets. This substrate held the highest proportion of thallus survivors. It was somewhat due to well ventilation allowing excess water to evaporate from lichen thalli. On the other hand, it assisted faster absorption of atmospheric water into thalli. Effect of this factor was shown by low growth rate. As water rapidly lost from thallus, photosynthetic period was shortened and consequently led to low growth rate. It is well recognized that thallus water content of lichen is related with atmospheric moisture (Armstrong, 1993; Fos, Deltoro, Calatayud, & Barreno, 1999; Green & Lange, 1991; Green et al., 2008; Green & Snelgar, 1981; 1982; Nimitr Osathanon, 2002). Therefore, tropical rain forest at KYNP which rain is frequent during the daytimes (Wetchasart Polyiam, 2005) enhanced lichen growth. Growth rate in rainy season was higher than dry season (see Figure 61).

Although the survived thallus fragments had high growth rate on plastic net, however, large proportion of death thallus was observed. This was probably related with size of the transplanted thallus fragments. In its natural habitat, thallus diameters smaller than 3 cm tend to disintegrate within 2 years by natural causes as well as biotic factor.

Nimitr Osathanon (2002) and Wetchasart Polyiam (unpublished, 2006) reported that *P. tinctorum* with thallus diameters less than 3 cm show signs of death and begin to disintegrate in 2 years, and all of the smaller thalli completely die in 6 years. Contrastingly, overall death of large thalli (diameter > 3 cm) was only 25% after the same period. Some of the larger thalli continue growing through their ninth years. A number of studies suggested that small thalli on plastic nets had higher rates of water loss. This was due to their low boundary layer resistance (Gauslaa & Solhaug, 1998) and thin cortex and medulla (Fos et al., 1999). As the transplanted thalli in the present study were small with diameters of only about 2 cm, water loss could be the cause of death.

Ceramic tile. This substrate is made from feldspar composing of several minerals calcium carbonate, sodium and aluminum. It has alkali property (Lee, Hodson, & Parsons, 1998; Lee & Parsons, 1999) and high water-holding capacity, which could supply water for lichen for longer period of photosynthesis. Two months after transplantation was the critical period for establishment of thallus fragments on new substrate. Large proportion of thalli transplanted to ceramic tile died during this period indicated that this substrate might not be suitable for growing lichen as previously believed.

Only seventeen thalli remained growing after 12 months of transplantation, however, they were grown over by the free-living algae *Scytonema* sp. This algal prefers alkaline substrates (Guiry, 2010). It grew fast in rainy season and completely covered the lichen and substrates in 25 month of transplantation.

Alkalinity and saturated substrate affect metabolism and growth of lichens (Armstrong, 1990b; Nash, 2008b). Armstrong (1990b) demonstrated that growth rate of *Xanthoria parietina* declines after wetting with alkaline buffer of calcium or magnesium carbonates. However, the disadvantage of this substrate was poor ventilation which favors growth of other epiphytes.

By contrast, growth rate of lichen on ceramic tile increased in dry season because lower moisture led to decreasing competition. Aptroot & van Herk (2006) reported that free-living algae grow well in the surrounding area of lichens under optimum humidity (Seaward, 2008, pp. 275-280). Nevertheless, thallus survivors on ceramic tiles had the highest growth rates than polycarbonate plates and plastic nets. However, growth rate of *P. tinctorum* on the three substrates were lower than that measured in natural habitats. (Nimitr Osathanon, 2002).

In the future if the ceramic tiles could be modified by adjusting pH, it might be the best substrate for transplantation to increase lichen production.

Effects of Angles of Inclination of Substrates on Survival and Growth of Isidia After Transplantation Under Shading Net in SF

This experiment was designed to find the best angles of inclination for germination of lichen diaspores. Transplantation of isidia of *P. sulfuratum* over nylon net installed at 0, 45 and 90 degree inclination in SF found that the highest germination of isidia occurred on 45 degree slope. Degree of slope affected rate of water drainage from surface, and consequently moistening period of lichen thallus and photosynthetic period of the inhabited lichen. This condition of this microhabitat is important on survival of tropical lichens under heavy rainfalls. However, Link and Nash (1984) reported that at 0^{o} (parallel with ground) slope of rock has strong influences on variability of crustose, foliose and fruticose lichens at Anaktuvuk Pass, Alaska. They found that foliose lichens dominated on the 30^{o} slopes. Substrate inclination also influences surviving and growing of various species (Armstrong, 1990a; Lawrey, 1980; Seaward, 2008; Wetchasart Polyiam, 2005).

In addition, *P. sulfuratum* produced longer rhizines to anchor on substrate, which was different from *P. tinctorum, P. praesorediosum* and *P. santi-angelii.* The latter three species grow on tree trunk in their natural habitat do not produce long rhizine.

Effects of 45° Inclined Nylon Substrate on Survival and Growth of Isidia and Soredia After Transplanted Under Shading Net in SF

This experiment was carried out after various factors that limited successful transplantation of vegetative propagules of lichens were elucidated in this study. This experiment was performed by transplantation isidia of *P. tinctorum* and soredia of *P. santi-angelii* on nylon net installed at 45 degree slope under shading net in SF. It was found that the soredia was more efficient than isidia on occupying this slope. The soredia germinated as many as 90% comparing with 70% of those found among the isidia. Furthermore, large number of weak isidia, 25-30%, deteriorated in rainy season. It was probably caused by delayed evaporation of its structure. Isidia is bound by cortex layer consisting of compact layer of mycobiont mycelium. By contrast, soredia lack of this layer, and thus evaporation is better than the former.

Moreover, a few samples of soredia and isidia grew almost completely covered the entire substrates after two years of transplantation (see Figure 66B and 67E). Therefore, 45° inclination of substrate was the best setting up for transplantation of lichen diaspores in order to achieve the highest production, whilst using the least amount of lichen resource.

Conclusions

This study underpinned the overriding importance of illumination that influenced lichen survivals in all tropical ecosystems studied. The main conclusions that can be drawn from this study are as follow;

1. Site specific species from the cool environment in the tropic barely survived in warmer climate, whereas some of those from the warm climate were able to adapt to cooler habitat. It indicated that lichens from high elevation in the tropic were sensitive to warm temperature and may be threaten to extinction. Whereas, those inhabited the warmer habitats probably migrate and grow in cooler environment of the higher elevation, only if the migration processes are not impeded.

2. Careful consideration must be taken for transplantation of vegetative propagules of lichens. Type of vegetative propagules, levels of tree trunk, aspect orientation and forest type, as well as transplantation periods interacted differently determined germination, survival and growth of the transplanted propagules.

Isidia and soredia were the most appropriate propagules for transplantation to get the highest number of juvenile lobe of lichens, about 60% of the transplanted materials survived in the LMF and SF after 10-20 months. Thereafter, they were encroached by natural enemies, which led to completely disintegrated of the both propagules in these forests.

Thallus fragment survived well in LMF at the canopy and mid trunk levels, and SF at the trunk base of the host trees. About half of the transplanted materials survived through the observation period of 60 months. The East and South facing trunks were the most appropriate sides for transplantation in LMF, and the Southern aspect in the SF (see Figure 40 and 41). Those from DEF and TRF could not support any development of thallus fragment in any aspect.

Therefore, LMF should be used as a nursery ground for germination of isidia and soredia to gain large amount of juvenile lobes, and later transfer to grow in SF to maximize biomass production.

3. All artificial substrates except glass bottle fail to supported development of the thallus fragments in the tree transplanted locations, LMF, TRF and SF. Lichens transplanted on wood, ceramic tile, concrete block, rubber tile, galvanized iron could not survived. Only glass bottle in all transplanted sites supported lichen materials. The bottles in TRF supported the highest proportion of survived thalli, and subsequently leaser in SF and LMF accounting for 24, 20 and 4 % respectively.

4. This study underpinned the prime important of illumination in all tropical ecosystem studied. Light intensity of 106-117 μmol $m^{-2}s^{-1}$ in early morning while lichen thalli were moistened, from absorbing atmospheric water during the night, was essential for development of the transplanted vegetative propagules of lichens.

5. The most successful transplantation of isidia and soredia occurred on 45^{o} inclined nylon substrate under shading net in SF. This technique required the least amount of lichen material and is most useful to enhance in situ production of lichens for conservation and sustainable utilization.

Recommendations

By using lichens which are sensitive to climatic condition as model organism, this study confirm the evidence that tropical species from high elevation are at risk of extinction by global warming. In order to conserve and utilize lichen resource, which produce unique natural products, sustainability under changing world. The following managements and further studies are essential:

1. Intensive studies on ranges of tolerance to habitat changes of endemic species from high elevation, which produce novel natural products.

2. Develop procedure and strategy to protect species from high mountain from threaten to extinction by climate change.

3. High elevation ecosystems such as LMF must be especially protected from other human activities to minimize synergistic impacts from climate change which is occurring sooner than expected.

4. In situ conservation of endemic and rare species is necessary. Transplantation by using vegetative propagule, especially isidia and soredia with proper techniques recommended in this study to appropriate habitats should be employed.

5. Studies on techniques for transplantation of other form of lichens, crustose and fruticose, to get the highest number of individual thallus and to gain the highest yield of biomass.