VEGETATIVE PROPAGATION OF RARE TREE SPECIES FOR FOREST RESTORATION

ANANTIKA RATNAMHIN

MASTER OF SCIENCE
IN ENVIRONMENTAL SCIENCE

THE GRADUATE SCHOOL
CHIANG MAI UNIVERSITY
MARCH 2010

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A THESIS SUBMITTED TO THE GRADUATE SCHOOL IN
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ABSTRACT

Forest restoration programs require production of high quality planting stock of a wide range of indigenous forest tree species. Because, many of these species have proved difficulties to propagate from seed, it is important to develop methods to produce planting stock via other means. The study, presented here, investigated vegetative propagation of trees from cuttings. The objectives of the research were i) to develop cutting propagation techniques, with simple, low-cost technology for tree species, which are rare or threatened with extirpation from northern Thailand and which have been difficult to grown from seed and ii) to test the effects of various treatments, including application of different of auxins and fungicides, leaf pruning, rooting media, and sources of cutting (positions in stem) in order to improve rooting success of the cuttings. This research was conducted in Doi Suthep-Pui National Park at the Forest Restoration Research Unit (FORRU) nursery. Nine rare tree species were selected for investigation: 1) *Crypteronia paniculata* Bl. var. *paniculata*, 2) *Diospyros coaetanea* Flet., 3) *Gardenia sootepensis* Hutch., 4) *Haldina cordifolia* (Roxb.) Rids., 5) *Ilex umbellulata* (Wall.) Loesn., 6) *Mesua ferrea* L., 7) *Rothmania sootepensis*

(Craib) Brem., 8) *Schoutenia glomerata* King ssp. *peregrine* (Craib) Roekm. & Hart., and 9) *Scleropyrum pentandrum* (Dennst.) Mabb. Five separate experiments were run; i) five concentrations and two forms of rooting hormones; namely control (without rooting hormone), Seradix® (powder containing IBA 3,000 ppm), IBA solution 3,000 ppm, IBA solution 8,000 ppm, and solution mixed of IBA and NAA 5,000:2,500 ppm (or 2:1) with all nine species, ii) three node positions of *Rothmania sootepensis*, iii) four treatments of fungicide; namely control (no fungicide), Benomyl, Captan, and Red lime with *Ilex umbellulata*, iv) leaf area treatments of leafless, trimmed half leaves, and full leaves of *Crypteronia paniculata*, and v) four propagation media; namely sand, sawdust, a mixture of sand and rice husk charcoal (1:1), and a mixture of sand, rice husk charcoal, and coconut husk (1:1:1) with *Mesua ferrea*.

None of these treatments were successful in producing viable planting stock in sufficient quantities, although limited success was achieved with *Shoutenia glomerata*. Nine percent of *Shoutenia glomerata* cuttings produced roots that grew up vigorously and long enough for potting. The best treatment was no hormone treatment (control), which produced the highest relative performance score (88.9%). However, survival of cuttings with both roots and shoots was low (4.7%) and roots emerged very slowly. It required almost 10 months from collecting cuttings to potting of rooted cuttings. Relative growth rate of cuttings of this species was very low. Therefore, this study found that it was not possible to produce viable planting stock of any of the species tested in less than one year from cutting propagation, using the treatments listed above.

ชื่อเรื่องวิทยานิพนธ์ การขยายพันธุ์แบบไม่อาศัยเพศของต้นไม้หายากเพื่อการฟื้นฟูป่า

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บทคัดย่อ

การฟื้นฟูป่าจำเป็นต้องมีการผลิตกล้าไม้ที่มีคุณภาพดีเป็นจำนวนมาก ซึ่งมักจะให้ครอบคลุมชนิดของพรรณไม้ท้องถิ่นหลายชนิด แต่มีพรรณไม้ท้องถิ่นหลายชนิดที่ผลิตกล้าไม้จากการเพาะเมล็ดได้ยาก ดังนั้นจึงมีความสำคัญเป็นอย่างยิ่งในการพัฒนาวิธีการผลิตกล้าไม้โดยวิธีอื่น ซึ่งวิธีทดสอบที่ใช้ในการศึกษาครั้งนี้คือการขยายพันธุ์แบบไม่อาศัยเพศโดยวิธีการตัดชำ โดยมีจุดประสงค์เพื่อ 1) พัฒนาวิธีการตัดชำด้วยขั้นตอนที่ง่ายและประหยัดต้นทุนในการผลิตกล้าไม้ท้องถิ่นชนิดหายากหรือใกล้สูญพันธุ์ในบริเวณภาคเหนือของประเทศไทย และประสบปัญหาในการขยายพันธุ์ด้วยเมล็ด และ 2) เพื่อทดสอบการชักนำให้ออกรากของกิ่งตัดชำจากปัจจัยต่าง ๆ คือ การใช้สารเร่งรากและยากำจัดเชื้อรา พื้นที่ใบ วัสดุตัดชำ และตำแหน่งของกิ่งตัดชำ งานวิจัยนี้ได้ทำการศึกษาในอุทยานแห่งชาติดอยสุเทพ-ปุย ณ เรือนเพาะชำหน่วยวิจัยการฟื้นฟูป่า ต้นไม้หายาก 9 ชนิดที่ทำการศึกษา ประกอบด้วย 1) กะอาม (*Crypteronia paniculata* Bl. var. *paniculata*) 2) ลำบิด (*Diospyros coaetanea* Flet.) 3) คำมอกหลวง (*Gardenia sootepensis* Hutch.) 4) ขว้าว (*Haldina cordifolia* (Roxb.) Rids.) 5) เน่าใน (*Ilex umbellulata* (Wall.) Loesn.) 6) บุนนาค (*Mesua ferrea* L.) 7) แสล่งหอมไก๋ (*Rothmania sootepensis* (Craib) Brem.) 8) รวงผึ้ง (*Schoutenia glomerata* King ssp. peregrine (Craib) Roekm. & Hart.) และ 9) ขี้หนอน (*Scleropyrum*

pentandrum (Dennst.) Mabb.) โดยแบ่งออกเป็น 5 การทดลอง ดังนี้ 1) ทดสอบกิ่งตัดชำต้นไม้ทั้ง 9 ชนิดกับ 5 ความเข้มข้นและ 2 ชนิดของสารเร่งราก ประกอบด้วย กลุ่มควบคุม (ไม่ใช้สารเร่งราก) เซราดิกซ์® (IBA 3,000 ppm รูปแบบผง) สารละลาย IBA 3,000 ppm สารละลาย IBA 8,000 ppm และสารละลาย IBA ผสม NAA ในอัตราส่วน 5,000:2,500 ppm (หรือ 2:1) 2) ทดสอบกิ่งตัดชำสแหล่งหอมไก่ 3 ส่วน คือ ส่วนยอด ส่วนกลาง และส่วนปลาย 3) ทดสอบกิ่งตัดชำเน่าในกับยาฆ่าเชื้อรา ประกอบด้วย 4 กลุ่ม คือ กลุ่มควบคุม (ไม่ใช้ยาฆ่าเชื้อรา) เบโนมิล แคปแทน และปูนแดง 4) ทดสอบกิ่งตัดชำกะอามกับพื้นที่ใบ 3 แบบ คือ ไม่มีใบ ครึ่งใบ และเต็มใบ และ 5) ทดสอบกิ่งตัดชำบุนนาคกับวัสดุตัดชำ 4 ชนิด คือ ทราย ขี้เลื่อย ทรายผสมแกลบ (1:1) และทรายผสมแกลบและขุยมะพร้าว (1:1:1)

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ABBREVIATIONS

ANOVA	:	one-way analysis of variance
cm	:	centimeter
cm^2	:	square centimeter
cm^3	:	cubic centimeter
CMU	:	Chiang Mai University
CPS	:	comparison among species
CRD	:	Complete Randomized Design
FORRU	:	Forest Restoration Research Unit
IAA	:	indoleacetic acid
IBA	:	indolebutyric acid
km	:	kilometer
km^2	:	square kilometer
LSD	:	least significant difference
m	:	meter
m.a.s.l.	:	meters above sea level
mm	:	millimeter
NAA	:	naphthaleneacetic acid
No	:	number
ppm	:	part per million
RGR	:	relative growth rate
RHGR	:	relative growth rate of height
RPS	:	relative performance score
RRGR	:	relative growth rate of root collar diameter
µg	:	microgram

CHAPTER 1
INTRODUCTION

Rationale

Tropical forests once blanketed the Earth like a wide green belt around the equator, with a special role in the conservation of biodiversity. They are the most ancient, the most diverse, and the most ecologically complex of land communities (Myers, 1992). Though occupying just only 7% of the earth land's surface, they are home to more than half the world's living plant and animal species (Wilson, 1988; Nawayuth, 2002). It has been estimated that a typical patch of tropical forests, just 6 km^2 contains as many as 1,500 species of flowering plant, 750 species of tree, 400 species of bird, 150 species of butterfly, 100 species of reptile, and 60 species of amphibian. The numbers of insects are so great that they can only be guessed at, but 0.01 km^2 may contain as many as 42,000 species (Lovejoy, 1989; Nawayuth, 2002).

Furthermore, tropical forests do far more than sustain biodiversity; they are home to indigenous peoples, pharmacopeias of natural products, and provide vital ecosystem services, such as flood amelioration and soil conservation. At regional and global scales, tropical forests also have a major influence on carbon storage and climate (Laurance, 1999a, Zimmer and Baker, 2009). They moderate the diurnal range of air temperatures and maintain atmospheric humidity levels. Forests absorb atmospheric carbon and replenish the oxygen in the air we breathe.

Although tropical forests contain many important natural resources, some of the most intensive land use and land cover changes are occurring in tropical countries (Alves, 2002; Arroyo-Mora *et al.*, 2005). Such changes are characterized by very rapid deforestation and a shift towards human-dominated landscapes (Laurance, 1999b; Houghton, 2002). The Food and Agricultural Organization of the United

Nations (FAO) estimated that an average of 154 thousand km^2 of tropical forest was destroyed each year, from 1980 to 1990 while another 56 thousand km^2 was logged (FAO, 1993). If we continue at the current rate of deforestation and destruction of major ecosystems like tropical forests and coral reefs, where most of the biodiversity is concentrated, we will surely lose more than half of all species of plants and animals on the earth by the end of 21st century (Wilson, 1992).

Deforestation is mainly caused by four key drivers: human population pressure, weak government institutions and poor policies, increasing trade liberalization, and industrial logging (Laurance, 1999a; Thomas *et al.*, 2004).

Similar processes are occurring in northern Thailand, a mountainous area with tropical ecosystems. This area has undergone considerable forest loss (Wangpakapattanawong and Elliott, 2008). According to the Royal Forest Department of Thailand (RFD, 2006), northern Thailand's forest cover was 68.54% (116,275.00 km^2) of the total land area in 1961, but by 2006 cover had dropped to 53.09% (88,368.11 km^2). A commercial logging ban since 1989 did not slow deforestation as much as expected, due to illegal logging, intensive agriculture system expansion, and development projects (Pragtong, 2000). Consequently, some indigenous forest tree species are now at high risk of extirpation or becoming rare, due to loss of habitat, particularly those with specific habitat requirements.

To ensure sustainability of these forests, there is therefore a need to conserve, regenerate, protect, and properly manage them. Tree planting by the government, non-governmental organizations, and local communities have been implemented on many areas of degraded forestland to help restore forests. These activities have been encouraged with by special events, for example, the King's birthday, the Queen's birthday, celebration His Majesty King Bhumibol Adulyadej's Golden Jubilee, etc. In the past, most reforestation projects involved planting monocultures of fast-growing commercially valuable tree species, such as teak, pine, and eucalyptus, since this was the quickest way to re-establish tree cover. However, such plantations are of low value for wildlife conservation, support low biodiversity, and do not create a self-

supporting ecosystem (Karimuna, 1995; Urbanska *et al.*, 1997; Ruiz-Jaen and Aide, 2005). The success of ecological restoration can be assessed in terms of gradually increasing levels of the following attributes: species richness and diversity indices of plants and animals, diversity of life forms, presence of keystone species, biomass and primary productivity, soil organic matter content, and moisture holding capacity (Elliott, 2000). Thus, planting trees should promote recovery of biodiversity and ecosystem structure and function.

One specialized form of reforestation is "forest restoration", which means re-establishing the original forest ecosystem that was present before deforestation occurred. This method involves planting tree species that play a vital role in the forest recovery create forest structure with multi-layered canopy, increase species diversity, and improve soil conditions (FORRU, 2008). This activity requires large-scale production of planting stock, of a wide range of indigenous forest tree species, but many of these species are difficult to propagate from seeds, due to long dormancy periods or seed production too late for seedlings to grow large enough by the optimum planting time (which is at the beginning of rainy season, mid June to mid July, in northern Thailand) (Vongkamjan, 2003).

Therefore, vegetative propagation from cuttings is one potential way to produce planting stock of such species. This technique has an advantage of low cost, low technology, ease of use, and rapidity (Hartmann *et al.*, 1990). It also produces uniform planting stock for large-scale forest restoration. Seedlings tend to grow to the suitable plantation size faster than those propagated from seeds. The length of time to first flowering and fruiting is also reduced (FORRU, 2005; Roh *et al.*, 2005).

Propagation from cuttings is increasingly being applied to a range of forest tree species to enhance the yield and quality of a wide range of forest products (Leakey, 2004). For example, for the threatened African pencil cedar (*Juniperus procera* Hoechst. ex Endl.) (Negash, 2002) and the threatened African wild olive (*Olea europaea* L. subsp. *Cuspidate* (Wall. ex DC.) Ciffieri) (Negash, 2003), cuttings propagation has proved to be an effective means of regenerating these valuable tree

species. On the other hand, cutting propagation could not be used successfully for the mass production of *Dryobalanops beccarii* and *Shorea macrophylla* (Khun and Dick, 1996) and *Shorea smithiana* (Subiakto *et al.*, 2005), which had low rooting percentages (17.0, 12.8, and 28.0-31.0%, respectively).

Successful propagation of tropical tree species by stem cuttings depends on many factors (Leakey, 1990), such as the composition of the propagation medium (Ofori *et al.*, 1996; Shiembo *et al.*, 1996; Mesén *et al.*, 1997) and different types or concentration of auxins (Kannark and Saiwa, 2000; Vongkamjan, 2003). In addition, in many species leaf area have a marked influence on rooting (Newton *et al.*, 1992; Khun and Dick, 1996; Ofori *et al.*, 1996; Shiembo *et al.*, 1996; Tarragó *et al.*, 2005), as same as position of the cutting (Mesén *et al.*, 1997; Wassner and Revetta, 2000; Trueman and Peters, 2006).

The aim of this research was, therefore, to investigate how to produce planting stock of rare tree species in northern Thailand by propagation from cuttings using a non-mist propagation system. In particular, these experiments were designed to assess the effects of application of different of auxins and fungicides, leaf pruning, rooting media, and sources of cutting (positions in stem) on rooting of cuttings, in order to determine appropriate treatments for mass production of these species.

Hypotheses

1. Propagation from cuttings is a suitable method to produce planting stock of rare tree species.
2. The effects of various treatments, including application of different of auxins and fungicides, leaf pruning, rooting media, and sources of cutting (positions in stem) on performance of cuttings vary among the tree species.

Research Objectives

1. To develop cutting propagation techniques for rare tree species that are difficult to grow from seed.
2. To test the effects of various treatments, including application of different of auxins and fungicides, leaf pruning, rooting media, and sources of cutting (positions in stem) on cuttings performance in terms of survival, vigour, and rooting.

Usefulness of the Research

This study should determine whether or not cuttings are a feasible and efficient way to produce planting stock of rare tree species which cannot be grown from seed. This should enable the inclusion of such tree species in forest restoration projects and ultimately help to prevent extinction of such tree species.

CHAPTER 2
LITERATURE REVIEW

Forest Loss in Northern Thailand

In the past, the northern Thailand was covered with dense forests. Despite their importance, data by the Royal Forest Department of Thailand (RFD, 2006) (Figure 2.1) showed that the percentage of forest-covered land in this region decreased greatly in recent years. Deforestation peaked in the 1970s, when the annual loss rate was about 3,695-3,756 km^2 (2.15-2.23%). The lowest loss of forest was in 1989 (180 km^2 or 0.1%), because a national ban on commercial logging was declared in that year in response to public concern and an outcry for more conservation-oriented policies to prevent forest degradation, which had been blamed for flooding and landslides in the south in 1988 (Pragtong, 2000). However, deforestation still continues.

The causes of forest loss include over-exploitation of forest resources, such as illegal logging, illegal trading of animal and plant species, shifting cultivation, over-hunting of wildlife, etc. (Office of Natural Resources and Environmental Policy and Planning, 2004). Moreover, infrastructure development and fire continue to degrade remaining forest patches (Wangpakapattanawong and Elliott, 2008).

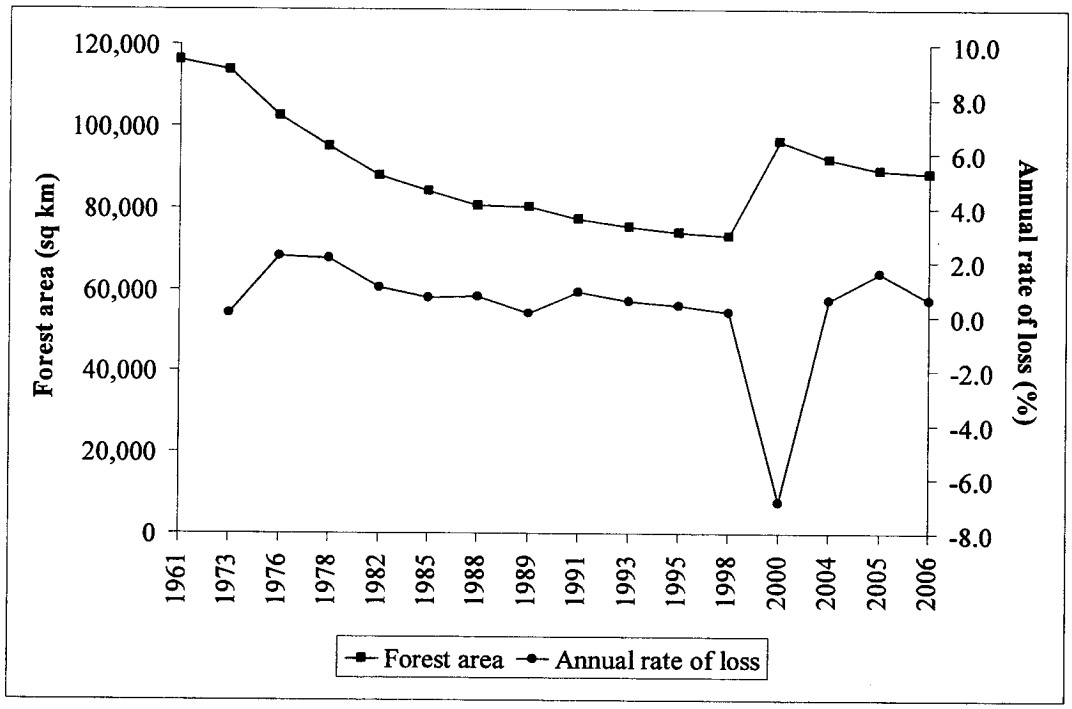

Figure 2.1 Forest area and annual rate of loss in northern Thailand between 1961 and 2006 (RFD, 2006)

Remark: 1) Northern Thailand's total area is 169,644.29 km^2.

2) Forest area acquired from satellite image processing of LANDSAT imageries. There is a change in scale of the interpret method from scale 1:250,000 to 1:50,000 since 2000. In addition, there was also a change in calculation method of remaining forest area from using Dot Grid to Geographic Information System (GIS) (Ongsomwang, 2002).

Thomas *et al.* (2004) studied the processes and dynamics of land use change in northern Thailand. They reported that the types of deforestation found in this region might be broken into three major components:

1) *Conversion of forest.* Initial conversion after 1960 throughout Thailand was associated with expansion of agriculture (Charuppat, 1998), both to feed the growing population and for exporting crops to provide funds for economic expansion. Conversion to agriculture was facilitated by heavy logging, and during the late 1970s, agriculture expansion combined with national security strategies further encouraged clearance of forests. Furthermore, some of non-agricultural land uses, such as resorts and golf courses, converted the land directly from forest.

2) *Logging of natural forest.* Logging helped fuel economic grow initially, but the combination of huge concession areas overlapping with protected forest areas and local communities, high official and unofficial harvest rates, low replanting rates, settlement and cultivation of logged areas, and slow expansion of plantation forests finally proved unsustainable (Pragtong and Thomas, 1990). Although logging concessions were stopped in 1989, illegal logging is still a problem in reserved forest and protected areas.

3) *Farmers in the forest.* In the mountains of north Thailand various ethnic minorities have long lived as 'farmers in the forest' (Kunstadter *et al.*, 1978). A web of often contested issues is associated with their land use practices, including opium production, shifting cultivation, rural poverty, and the impact of their land use practices on protected forest areas and environmental services.

Impacts of Deforestation in Northern Thailand

Deforestation is the one of the most important environmental problems because it is the main cause of rapid biodiversity loss. Furthermore, it also impacts on the human population and environment including; ecological effects (environmental change, soil condition, soil erosion, siltation, and water balance) as well as economic,

social, and political effects (Bhumibhamon, 1986). In addition, climate change and global warming, partly due to forest loss, are now a serious concern that has aroused widespread attention.

With the world-wide increase in concern about global climatic change and the rate of destruction of tropical forests, attention has focused on the influence of regional and global climates and the impact that large-scale forest clearance having on them. Many of the uncertainties involved in attempting to assess and model the impact of tropical forests clearance have been pointed out.

Henderson-Sellers (1987) concluded that tropical forest clearance might contribute to regional or global climate change in four main ways:

1) Affecting atmospheric composition
2) Affecting evaporation-rainfall patterns through its impact on the hydrological cycle
3) Changing in surface albedo
4) Affecting atmospheric turbulence by causing change in the aerodynamic roughness of the vegetation canopy by adding dust particles to the atmosphere as a result of biomass burning and increased wind-blown dust from drier and more exposed soil surfaces

In mountainous northern Thailand, the consequences of deforestation are particularly prominent, since it results in reducing quality of life, as watersheds become eroded, flash floods occur in rainy seasons, streams dried up in dry seasons, and rivers become choked with silt. Large mammals, such as elephants, tigers, bears, and wild cattle have been mostly extirpated and populations of smaller animals, such as gibbons and hornbills have become perilously small and isolated (Kerby *et al.*, 2000).

Forest Restoration in Northern Thailand

As a response to deforestation, trees have been planted to restore many areas of deforested or degraded land back into forest. National reforestation projects were initiated in 1994 by the Royal Forest Department of Thailand (RFD), which is responsible for managing all forest resources in Thailand. They established monocultures of common economic trees and fast-growing non-native species (mostly pines, teaks, and eucalyptus) for economic forestry. However, such plantations are not so useful in term of biodiversity conservation value, because they are a poor substitute for the original forest and cannot replace forest ecosystems, which have high complexity of ecological function and structure (Karimuna, 1995). Therefore, attitudes towards reforestation have recently changed considerably resulting demands to develop effective forest restoration methods to provide a viable alternative to such plantations (FORRU, 2005).

Forest Research Restoration Unit (FORRU, http://www.forru.org) is a joint initiative between Biology Department, Faculty of Science, Chiang Mai University (CMU) and Doi Suthep-Pui National Park (under the Thailand Government's Department of National Parks, Wildlife, and Plant Conservation) to promote research on forest restoration in northern Thailand (Elliott, 2001; FORRU, 2005). Since November 1994, the aim of the unit has been to develop effective methods to complement and accelerate natural forest regeneration on deforested sites within conservation areas, to increase biodiversity and protect watersheds. Since species selection step is very important for success of any forest restoration program (Elliott *et al.*, 2003), FORRU has been successfully developing and adapting the "Framework Species Method" to restore evergreen forest on abandoned agricultural fields in an upper watershed at 1,300 m.a.s.l. in Doi Suthep-Pui National Park, Chiang Mai province.

The "Framework Species Method" originated in northern Queensland, Australia to repair damaged tropical rain forest (Goosem and Tucker, 1995) by planting a mixture of 20-30 indigenous tree species in a single step. Framework tree

species are selected that are fast growing with an ability to shade out competing weeds and attract wildlife into planted areas. Subsequently, biodiversity is restored when the planted framework trees attract seed-dispersing animals by produce resources (e.g. fruits, nectar-rich flowers or bird nest sites, etc.). Seed-dispersing animals transport seeds of many additional tree species from nearby natural forest into planted sites, which restores the forest structure and function to its original condition, thus accelerating the return of biodiversity (FORRU, 1998; Elliott *et al.*, 2003; FORRU, 2005; Wangpakapattanawong and Elliott, 2008).

These days, restoring forests by planting a wide range of indigenous forest trees is recommended for reforestation projects because it can help to promote biodiversity (Karimuna, 1995; Jantakad and Gilmour, 1999; Xueying *et al.*, 2000; FORRU, 2008).

Vegetative Propagation by Cuttings

Forest restoration requires a continuous supply of high quality planting stock of a wide range of native forest tree species. Although research on propagation of commercial species is well advanced, forest restoration involves planting lots of species, which have never been grown before. Information on how to propagate native forest trees is essential to the process (FORRU, 2000). Unfortunately, many native tree species cannot be grown from seeds. For example, seed production is hampered by unpredictable fruiting seasons, irregular fruiting, recalcitrance of the seeds, and attack by insects and other animals. Therefore, propagation of some tree species from seeds is difficult. Consequently, vegetative propagation offers an important solution to provide a reliable supply of planting stock (Kaosa-ard *et al.*, 1998; Kannark and Saiwa, 2000; Priadjati *et al.*, 2001; Subiakto *et al.*, 2005).

However, vegetative propagation could contribute to genetic erosion, which is defined as the loss of genetic diversity and commonly refers to the reduction in the quantities of specimens of a species (Solbrig, 1991). Genetic diversity is important to a species' fitness, long-term viability, and ability to adapt to changing environmental

conditions. Also, plant populations that are less genetically diverse may be more susceptible, in some cases, to pathogens or other environmental stresses. Genetically eroded populations may be less competitive with introduced invasive species. Overall, genetic erosion can have cascading effects throughout the ecosystem (Genetic Resources Conservation Program, 2006).

On the other hand, all the endangered species are plagued by varying degrees of genetic erosion and most need to keep their population viable and to keep them from going extinct in the long run. The more critically endangered the species is (the smaller the population is), the more magnified the effect of genetic erosion gets when each surviving individual of the species is lost without getting a chance to propagate (Acquaah, 2007).

Vegetative or asexual propagation means an increasing of tree numbers by duplication of a whole plant from any living organ, such as a portion of root, stem, or leaf, induced to form roots and shoots by rooting hormone, chemical, mechanical, and/or environmental manipulation (Mahlstede, 1966; Hartmann *et al.*, 2002; Thompson, 2005).

Use of cuttings is an easy vegetative propagation method that can rapidly produce a lot of trees for planting, which fruit early in life (an important framework species criterion). Any portion of a plant, separated from the parent plant for propagation purposes, is usually described as a "cuttings" (Wright, 1973). Propagation from cuttings has several advantages over germinating plants from seeds, including handing of relatively small numbers of many different species, saving time and labor, and it is also inexpensive and easier to practice than other propagation methods (Hartmann *et al.*, 1990; Kantarli, 1993; FORRU, 2005; Roh *et al.*, 2005). Therefore, the principle of cutting propagation is to cut a part of a tree and put the cutting into an appropriate medium and growing environment, to promote rooting, shooting, and growing of a new tree (Mahlstede, 1966; Meka, 2006). However, a tree produced from a cutting will be a genetically identical "clone" of the tree from which the cutting was collected. This presents difficulties for conservation which seeks to

retain genetic diversity, particularly within small populations of rare or endangered species, which may be undergoing genetic drift.

Cuttings are classified into four general categories: roots, stems, leaves, and specialized structures, such as tubers and rhizomes (Mahlstede, 1966). Stems are the most convenient and popular method of propagation both in conservation and commercial situations (Wright, 1973, Matthews, 1999). Furthermore, stem cuttings can be used to produce a continuous supply of planting stock throughout the year for forest restoration activities. For example, in Indonesia, vegetative propagation of dipterocarps, through stem cuttings, has been developed at the Wanariset Research Station since 1987, as a feasible technique which is generally accepted and applied by forest concession holders throughout the country (Priadjati *et al.*, 2001).

In Thailand, very little work on cuttings has been carried out on native forest tree species. For example, Vongkamjan (2003) developed a novel cutting propagation technique, with simple, low-cost technology for ten native tree species and resulted as only five of ten tree species achieved a maximum of 60% or more cuttings developing roots. Up till now, the development of this technology has focused largely on exotic and commercial plantation trees species (e.g. acacias, eucalypts, and teak) (Blakesley *et al.*, 2000) together with horticulture crops. For example, for teak, there have been many studies on cuttings (Rattanawatkul and Wattanasuksakul, 1996; Kaosa-ard *et al.*, 1998).

Rattanawatkul and Wattanasuksakul (1996) studied cuttings of teak (*Tectona grandis* Linn.). They developed a rapid system based on cuttings for mass production of planting stock at a reasonable cost. The cutting propagules with shoot buds aged 3-5 months old were better than first and second internodes. Media composition has no significant effect on root initiation. Applying indolebutyric acid (IBA) (concentration level between 0-300 ppm) also had no significant effects, so they concluded that there was no need to use the hormone if cuttings were being propagated during the rainy season.

Factors Affecting Cuttings

Effects of Species

Great differences exist among plants of different species in the rooting ability of cuttings taken from them. Stem cuttings of some species root so readily that the simplest facilities and care give high rooting percentages. Cuttings of some difficult species can be rooted only if various influencing factors are taken into consideration and maintained at the optimum condition (Hartmann *et al.*, 1990).

The age of the parent tree from which cuttings are derived has a significant effect on rooting response. Several studies have shown that cuttings taken from juvenile stock plants form roots faster than those taken from mature plants (Mahlstede, 1966; Kaosa-ard *et al.*, 1998; Wassner and Ravetta, 2000; Negash, 2002; Kibbler *et al.*, 2004; Tarragó *et al.*, 2005). This was true whether comparing juvenile and mature material from a range of stock plants or from the same tree (Kibbler *et al.*, 2004).

Negash (2002) reported that the most critical factor in the vegetative propagation of the threatened African pencil cedar (*Juniperus procera* Hoechst. Ex Endl.) was the age of the stock plants. Cuttings obtained from 5-month-old seedlings rooted nearly twice as fast as those derived from 15-month-old stock plants. For example, 50% of cuttings derived from 5-month-old stock plants rooted within 9 weeks after treatment, whereas it took 18 weeks to attain a comparable percentage rooting for cuttings derived from 15-month-old stock plants.

Tarragó *et al.* (2005) studied adventitious rooting of *Ilex paraguariensis* cuttings. Softwood cuttings, harvested from young 3-year-old plants and adult 20-year-old plants, were rooted under intermittent fog. They reported that the mean rooting of cuttings from the juvenile materials (3-year-old plants) was nearly 100%, while the adventitious rooting of the adult materials (20-year-old plants) varied between 17 and 55%.

Kibbler *et al.* (2004) studied the effects of maturation on rooting of cuttings of *Backhousia citriodora* F. Muell. To determine that juvenility, and not the position of the cutting on the stock plant, affected rooting, 10 mature plants, propagated from cuttings and over 2 m in height were sampled. For each of these 10 plants, 5 cuttings taken from the base of the plant were compared with 5 cuttings taken from the apex. They found that the juvenile and mature materials taken from the same tree were significantly different. After 24 weeks, the rooting ability of the cuttings taken from the mature materials was 40% while that of the juvenile materials was higher than 90%.

Moreover, in difficult-to-root woody plant species, ease of adventitious root formation declines with age of the parent tree, resulting in a propagation problem since desirable characteristics are frequently not expressed until after a plant has reached maturity (Hartmann *et al.*, 1990).

It has long been known that the presence of leaves on cuttings exerts a strong stimulating influence on adventitious root initiation (Weaver, 1972; Hartmann *et al.*, 1990; Newton *et al.*, 1992; Khun and Dick, 1996; Ofori *et al.*, 1996; Shiembo *et al.*, 1996; Tarragó *et al.*, 2005) in terms of a supply of auxins and nutritional factors (Jarvis, 1986; Gaspar *et al.*, 1997). Plant propagators are well aware that loss of leaves from cuttings greatly reduces the chances of successful rooting (Weaver, 1972). Moreover, the size of leaves retained is also influential in many species (Ofori *et al.*, 1996; Shiembo *et al.*, 1996).

Khun and Dick (1996) studied vegetative propagation from cuttings of 3 indigenous timber species, including *Shorea macrophylla*, *Gonystylus bancanus* and *Drybalanops beccarii*. All cuttings were treated with growth hormone Seradix® #3 and placed in the same rooting medium (sand and pebbles). *Gonystylus bancanus* achieved the highest rooting rate (51.7%), but rooting was less prevalent for *Drybalanops beccarii* (17.0%) and *Shorea macrophylla* (12.8%). None of the leafless cuttings in this study produced roots. Therefore, stem cuttings of these dipterocarp species must retain leaves for successful root initiation and development.

Furthermore, for *Ilex paraguariensis*, percentage rooting and root number were positively correlated with the presence of leaves on the cuttings (Tarragó *et al.*, 2005).

Shiembo *et al.* (1996) studied the effects of leaf area on rooting percentage and number of roots per rooted cutting of leafy stem cuttings of *Irvingia gabonensis* in a non-mist propagation system in Cameroon. Five leaf area treatments, namely 0, 12.5, 25, 50, and 80 cm^2 were tested. Leaf area had a pronounced effect on rooting percentage, with the highest values recorded in the 80 cm^2 treatment at weeks 3 and 5. At week 3, none of the leafless cuttings rooted, and week 5, all of the leafless cuttings died. The results of this experiment indicated that *Irvingia gabonensis* may be successfully propagated by leafy stem cuttings retaining at least 80 cm^2 of leaves.

Ofori *et al.* (1996) reported that the effect of leaf area on final rooting percentage was highly significant in *Milicia excelsa* by stem cuttings. Total defoliation of the cuttings drastically reduced rooting percentage but did not prevent it altogether. However, no statistical differences in rooting percentage were recorded between the cuttings with leaf areas of 20, 40, and 60 cm^2. However, the mean number of root per rooted cutting increased with increasing leaf area to 40 cm^2. Therefore, cuttings with a leaf area of around 40 cm^2 should be used for mass production of *Milicia excelsa* cuttings.

Atangana *et al.* (2006) investigated rooting ability and the effects of leaves on juvenile cuttings of *Allanblackia floribunda* for different leaf areas (0, 12.5, 25, and 50 cm^2). Leafy cuttings (12.5, 25, and 50 cm^2) started to root 10-12 weeks after the experiment was set up, while leafless cuttings did not root throughout the experiment (38 weeks). However, leaf area in the leafy cuttings did not affect rooting ability at 38 weeks (14.8 ± 1.69, 13.3 ± 1.57, and 13.3 ± 1.44% for 50, 25, and 12.5 cm^2, respectively). This suggested that for vegetative propagation of *Allanblackia floribunda*, some leaves should be left on the cutting but the leaf area is not important.

Percentage rooting is also usually strongly correlated with cutting length (Leakey *et al.*, 1985), which is normally expressed as the number of nodes. Generally

4-6 nodes and 10-15 cm long cuttings provided good propagation material for all species (Kantarli, 1993). Extremely thin or woody cuttings should be avoided (Khun and Dick, 1996).

Moreover, cutting materials must be free of diseases and insect pests. Treatments with fungicides prevent fungal infection and result in both better survival in the cuttings and improved root quality (Mahlstede, 1966; Hartmann *et al.*, 1990; Matthews, 1999).

Rooting Media

The importance of propagation medium for the rooting of leafy cuttings is widely recognized. In general, an appropriate rooting medium is described as one with an optimal volume of gas-filled pore space and an oxygen diffusion rate adequate for respiration. Provision of sufficient water to prevent wilting is also a prime requirement. Moreover, the medium must hold the cuttings in place during the rooting sequence and be inexpensive, readily available, uniform, long lasting, inert, and free from diseases and toxic substances (Mahlstede, 1966; Matthews, 1999; Hartmann *et al.*, 2002; Thompson, 2005).

Materials are used as rooting media, including sand, rice hush charcoal, coconut husk, coffee compost, coir dust, rice husk, virgin soil, top soil, sawdust, and mixture of these in various proportions (i.e. sand:coconut husk, sand:coffee compost, sand:rice husk charcoal:coconut husk, etc.). There is no universal or ideal rooting mix for all cuttings. The optimum propagation medium to be used depends on the plant species, cutting type, season, type of propagation system, cost, and available of the medium components (Hartmann *et al.*, 1990).

Ofori *et al.* (1996), Shiembo *et al.* (1996), and Mesén *et al.* (1997) reported that tree species differ in their response to different media. Cuttings of *Irvingia gabonensis* (Shiembo *et al.*, 1996) and *Milicia excelsa* (Ofori *et al.*, 1996), had higher rooting percentages in sawdust than in other media tested, contrasting with the results

obtained with *Cordia alliodora* (Ruiz & Pavon) Oken for which both the rooting percentage and the number of roots per cuttings were reduced when sawdust was used (Mesén *et al.*, 1997).

Free (1957) and Rattanawatkul and Wattanasuksakul (1996) also reported that the cuttings of many species root successfully in a variety of rooting media. For example, there were no significant differences in root initiation of the cuttings of teak (*Tectona grandis* Linn.) between sand, burnt rice husk, sand:burnt rice husk (1:1), and sand:burnt rice husk:coconut coir (1:1:1) (Rattanawatkul and Wattanasuksakul, 1996).

Sand is often used for propagation. Just plain sand works well with a large number of species (Free, 1957; Atangana *et al.*, 2006) because sand improves aeration, wetting, and flow ability (Ahmad and Hamzah, 1993). However, the chief disadvantage of sand is its great weight and it holds little water (Free, 1957). Therefore, it is used mostly in combination with other materials (Hartmann *et al.*, 1990). Sand and rice husk charcoal combined, equal part of each by volume, is a good medium for cuttings in Thailand (Vaddhanaphuti, 1999). Rice husk charcoal is a lightweight material, improves aeration, and holds a minimal amount of water. Moreover, these two materials are low in cost and available all year round.

Since, there are many conflicting reports in regard to the best medium for rooting cuttings, each cutting type has a set of optimum conditions under which rooting will take place. Properly handled, cuttings will root satisfactorily in a wide range of media (Mahlstede, 1966).

Environmental Conditions

Environmental conditions are pivotal factors for the success of cuttings propagation (Kantarli, 1993; Atangana *et al.*, 2006). Essential conditions for rooting cuttings are the presence of moisture, air, and warmth. Cool air and high humidity at the leaf surfaces minimize water loss from the material, while a moist, warm rooting medium encourages fast root development (Matthews, 1999).

In the case of leafy cuttings, loss of moisture should be reduced to a minimum (Grange and Loach, 1983). The need for moisture is obvious, because the cutting has been isolated from its former water supply and is in danger of being desiccated. This applies particularly to leafy cutting, as their leaves are still transpiring moisture (Wright, 1973). Therefore, a saturated atmosphere is usually achieved by keeping the cuttings in a close shade frame and maintaining a humid atmosphere or by mist. The aim of misting is to maintain continuously a film of water on the leaves, thus reducing transpiration and keeping the cutting turgid until rooting can take place (Wright, 1973; Grange and Loach, 1983).

Rooting of cuttings is usually accelerated by heat both from air and rooting media. However, if the air temperature is too high, most of the stored food in cutting stems would be rapidly utilized for shoots growth leaving little energy for root development (Wright, 1973). High air temperature tends to accelerate bud development in advance of root development and to increase water loss from the leaves (Hartmann *et al.*, 1990). An important rule, however, is that the air temperature should be below that of the medium in which the cuttings are inserted. This means that while callus production and rooting are forwarded, the development of the buds and leaves is not so rapid (Wright, 1973). Therefore, it is important that adequate moisture status be maintained around cuttings, otherwise desiccation will inhibit root formation. And as the cuttings have no roots to make good the loss of moisture from their leaves may caused them to shrivel up and die. The consensus regarding the optimum medium temperature for propagation is 25-32°C for tropical species (Hartmann *et al.*, 1990; Vaddhanaphuti, 1999). Daytime air temperature of about 21-27°C with night temperatures about 15°C are satisfactory for rooting cuttings (Samanond, 1983; Hartmann *et al.*, 1990; Na Nongkhai, 1999).

Photosynthesis must be maintained in cuttings to produce carbohydrate for root formation (Wright, 1973; Davis, 1988; Newton and Jones, 1993b; Thompson, 2005). Therefore, light is needed by cuttings with leaves, to provide the energy through photosynthesis, on which plant metabolism depends (Thompson, 2005). However, when leafy cuttings are inserted in a frame they usually require shade from

bright light to prevent wilting. Shading reduces over-heating and desiccation; it also reduces light levels and delays results. If shaded continuously, however, the leaves are unable to manufacture food – light is essential for this process – and the cuttings may die of starvation (Wright, 1973). Finding ways to combine high light levels with high atmospheric humidity and preventing overheating is the key to success with cuttings.

Misting inside clear polyethylene under black shade netting (approximately 30% sunlight) helps to control all the above mentioned environmental conditions at a level suitable for rooting of cuttings to photosynthesizing food as its allows a cool moist leaf surface at all time and helps maintain high humidity at higher temperatures. This propagation system is relatively cheap and easy to maintain, and is, therefore, highly appropriate for use in rural tropical areas (Newton and Jones, 1993a). It is important that only a minimum amount of water should be used. This is because excessive water leaches out nutrients from the compost which may cause starvation. Moreover, a directly injurious effect on the cuttings can occur from over-watering (Wright, 1973). In addition, water quality is an important factor in rooting cuttings. For good results, the available water supply should not contain total soluble salts in excess of 1,400 ppm. The salts are combinations of such cations as sodium, calcium, and magnesium with such anions as sulfate, chloride, and bicarbonate. Water containing a high proportion of sodium to calcium and magnesium can adversely affect the physical properties and water-absorption rates of soils and should not be used for irrigation purposes (Hartmann *et al.*, 1990).

The conditions that are suitable for rooting leafy cuttings, that is warmth and moisture, are also very favorable to certain plant diseases. Strict hygiene is, therefore, necessary in propagating frames. The frame should be also examined frequently and any dead leaves carefully removed (Wright, 1973).

Fungicides

The most common risk associated with vegetative propagation by cuttings is that of fungal infection. Therefore, fungicide should be applied. Once cuttings are collected, they should be selectively treated with broad-spectrum fungicidal dips, prior to sticking, and/or chemical drenches during propagation (Hartmann *et al.*, 2002).

Benomyl is the most widely used fungicide for propagation and ornamental use (Kannark and Saiwa, 2000; Hartmann *et al.*, 2002). Moreover, some commercial substitutes for Benomyl, include Bavistin (Pakanisamy and Subramanian, 2001) and Captan (Tarragó *et al.*, 2005), together with Caocobre and Ridomil Plus (Atangana *et al.*, 2006). For Thailand, red lime also famous used with cuttings to prevent fungal infection by coating it on to the base of cuttings after application of rooting hormone and before putting cuttings into the rooting medium (Meefun, personal communication).

On the other hand, no serious pests and diseases problems associated with large dipterocarps have been experienced so far. Some species even showed the presence of insecticides and fungicides in their resins (Priadjati *et al.*, 2001).

Rooting Hormones

The purpose of using rooting hormones is to stimulate root and shoot initiation (Nilsamranchit, 1994), increase high rooting percentage and number of roots, develop rooting system, and generate consistent rooting (Vaddhanaphuti, 1999). All classes of growth regulators – auxins, cytokinins, gibberellins, ethylene, and abscisic acid, as well as ancillary compounds, such as growth retardants/inhibitors, polyamines, and phenolics – influence root initiation either directly or indirectly. However, the ability of auxins to promote adventitious root development in leafy stem cuttings is well known (Wright, 1973; Leakey, 1990; Hartmann *et al.*, 1990; Kannark and Saiwa,

2000; Guo *et al.*, 2008), and has been attributed to enhance transport of carbohydrate to the base of the cutting (Hartmann *et al.*, 1990; Hartmann *et al.*, 2002).

Auxins are plant hormones that stimulate initiation of adventitious root formation and root development including an increase in quality and higher even rooting (Wright, 1973; Kannark and Saiwa, 2000; Guo *et al.*, 2008). This means that plants, raised from auxin-treated cuttings can often be planted out earlier and will usually grow faster than plants from non-treated cuttings (Wright, 1973). Indoleacetic acid (IAA), indolebutyric acid (IBA), and naphthaleneacetic acid (NAA) are the most commonly used rooting hormones. Two synthetic forms of auxin that available commercially, IBA and NAA, are reliably promoted rooting in cuttings than IAA because IAA is very unstable in plants and its decomposition occurs rapidly in unsterilized solutions (Weaver, 1972). Both of these chemicals are available in liquid, talc, tablet, and gel formulations (Blythe *et al.*, 2004). However, NAA is more toxic than IBA. Therefore, IBA is widely used because it is non-toxic to most plants over a wide range and promotes root growth in a large number of plant species (Weaver, 1972; Vaddhanaphuti, 1999). Furthermore, mixed IBA and NAA induce a higher percentage of cutting to root in some species than either material used alone (Hartmann *et al.*, 2002). The acid form of auxins is relatively insoluble in water, but can be used a few drops of alcohol (isopropyl, ethanol, methanol), acetone, dimethyl sulfoxide to initially dissolve before adding to water. The use of old chemical solutions of rooting hormones to aid rooting of cuttings has sometimes produced negative results. Therefore, fresh preparations of hormone should be used for better results (Weaver, 1972; Hartmann *et al.*, 1990; Hartmann *et al.*, 2002).

Commercial root-promoting chemicals are normally applied to the basal portion of cuttings using a liquid or talc formulation of auxins. The quick-dip method is often preferred by commercial propagators for application of liquid auxins formulations for reasons of economy, speed, ease, and uniformity of application and results. An extended basal soak may be utilized for some difficult-to-root species (Hartmann *et al.*, 2002). However, cuttings of some species root readily without an auxin treatment, while cuttings of other species benefit from auxins treatment through

enhanced promotion of rooting; benefits may be dependent upon the species and cultivar, condition of the cutting wood, time of year, and other factors (Hartmann *et al.*, 2002). Moreover, in same tree species, the rooting response varies with the auxin type, auxin concentration, and different method of auxin application (Kannark and Saiwa, 2000; Blythe *et al.*, 2004; Guo *et al.*, 2008).

Trueman and Peters (2006) studied the requirement of applied auxin to induce rooting in tip cuttings and lower segment cutting of Wollemi pine (*Wollenmia nobilis* W.G. Jones, K.D. Hill & J.M. Allen). They found that IBA application (at 1,500, 3,000, or 8,000 ppm) had no effect on final rooting percentages. Both types of cuttings proved easy-to-root, with mean rooting of 71% for tip cuttings and 82% for lower segments.

Shiembo *et al.* (1996) studied the effect of IBA concentration on rooting of leafy stem cuttings of *Irvingia gabonensis*. Five IBA concentrations, namely 0, 8, 40, 200, and 250 µg IBA dissolved in 0.01 cm^3 of alcohol were tested. Application of IBA was unnecessary for the successful propagation, either in terms of root number or rooting percentage, either root development was more rapid in the 200 and 250 µg treatments.

Moreover, successful rooting without application of auxin has been reported in a number of tropical tree species, such as *Milicia excelsa* (Ofori *et al.*, 1996), *Nauclea diderrichii* (Leakey, 1990), and *Allanblackia floribunda* (Atangana *et al.*, 2006).

Vongkamjan (2003) reported that the effects of various hormone treatments on leafy stem cuttings varied among the species tested. Seradix® #2 (powder contained IBA 3,000 ppm) produced the best results with *Eurya acuminata*, *Ficus lamponga*, and *Ficus hirta*, while Seradix® #3 (powder contained IBA 8,000 ppm) was best with *Debregeasia longifolia* and *Saurauia roxburghii*. Solution IBA 8,000 ppm produced the best results with *Colona flagrocarpa* and *Morus macroura*. Solution IBA:NAA in ratio 1:1 was the best with *Macaranga kurzii* and solution IBA:NAA in ratio 2:1 or

solution IBA 3,000 ppm with *Ficus superba*. However, *Morus macroura* cuttings grew roots most efficiently without any hormone treatment.

Kannark and Saiwa (2000) studied stem cuttings from wild seedlings in *Amoora polystachya*. Both auxins (IBA and NAA) and concentrations of auxin affected to root formation for the cuttings. IBA seemed to be the best root promotion over NAA. They suggested that IBA with the concentration 1,000 ppm was the best root promotion for stem cuttings in wild seedlings of *Amoora polystachya*.

Blythe *et al.* (2004) evaluated rooting and initial shoot growth from application of auxin on leafy stem cutting propagation of four tropical species. Some species responded as well to a foliar application (spray treatment) of auxin as to a basal quick-dip, while the standard basal quick-dip or no auxin treatment continued to be preferable with many other species. Stem cuttings of *Aglaonema modestum* with basal quick-dip is of benefit for rooting of this species. Basal quick-dip may provide some improvement in rooting of *Ficus benjamina* compared to untreated cuttings, but the improvement may be small, while spray applications of auxin do not appear to be of benefit. The best overall rooting on cuttings of *Gardenia augusta* 'Radicans' was obtained using a foliar spray or a basal quick-dip in comparison to untreated cuttings. However, an auxin may be unnecessary for *Hedera helix* 'Ivalace'. Subsequent shoot or root development on cuttings of all species receiving the spray treatments was similar in most cases to cuttings receiving no auxin treatment or a basal quick-dip treatment.

As with other agricultural chemicals, governmental regulations generally mandate employee safety training prior to the use of root-promoting chemicals and use of personal protective equipment (chemical-resistant gloves, eye protection, appropriate clothing) during the preparation and use of these chemicals. Process modifications that permit a reduction in the time required to apply the root-promoting chemicals may result not only in cost savings, but also an increase in employees' sense of safety, comfort, and health (Blythe *et al.*, 2004).

CHAPTER 3
STUDY SITE

General Description

This study was conducted in Doi Suthep-Pui National Park, Chiang Mai, northern Thailand (Figure 3.1). The Park was established on 14 April 1981 under the jurisdiction of the Royal Forest Department of Thailand (RFD) and is now managed by the Department of National Parks, Wildlife, and Plant Conservation. It is situated directly west of Chiang Mai City at 18° 50′ north altitude and 99° 00′ east altitude and included an area of 261 km^2. The main part of the national park is Doi Suthep and Doi Pui, which are N-S aligned mountains rising from 350 m.a.s.l. to 1,610 m.a.s.l. (Doi Suthep) and 1,685 m.a.s.l. (Doi Pui). Base rocks are mostly granite, and soils are generally deep and highly weathered. There are 2 main types of forest in the park, including deciduous forest (from the lowlands up to about 950 m.a.s.l.) and evergreen forest (from about 950 m.a.s.l. to the summit of Doi Pui, 1,685 m.a.s.l.) (Maxwell and Elliott, 2001).

The nursery experiment was carried out at the Forest Restoration Research Unit (FORRU) Nursery, which was established in 1994 to develop methods to restore forest ecosystems on degraded areas for conservation of biodiversity (Figure 3.2). The nursery is situated near the accommodation centre of Doi Suthep-Pui National Park at approximately 1,050 m.a.s.l. in a primary evergreen, seasonal, hardwood forest on granite bedrock (Kuarak, 2002).

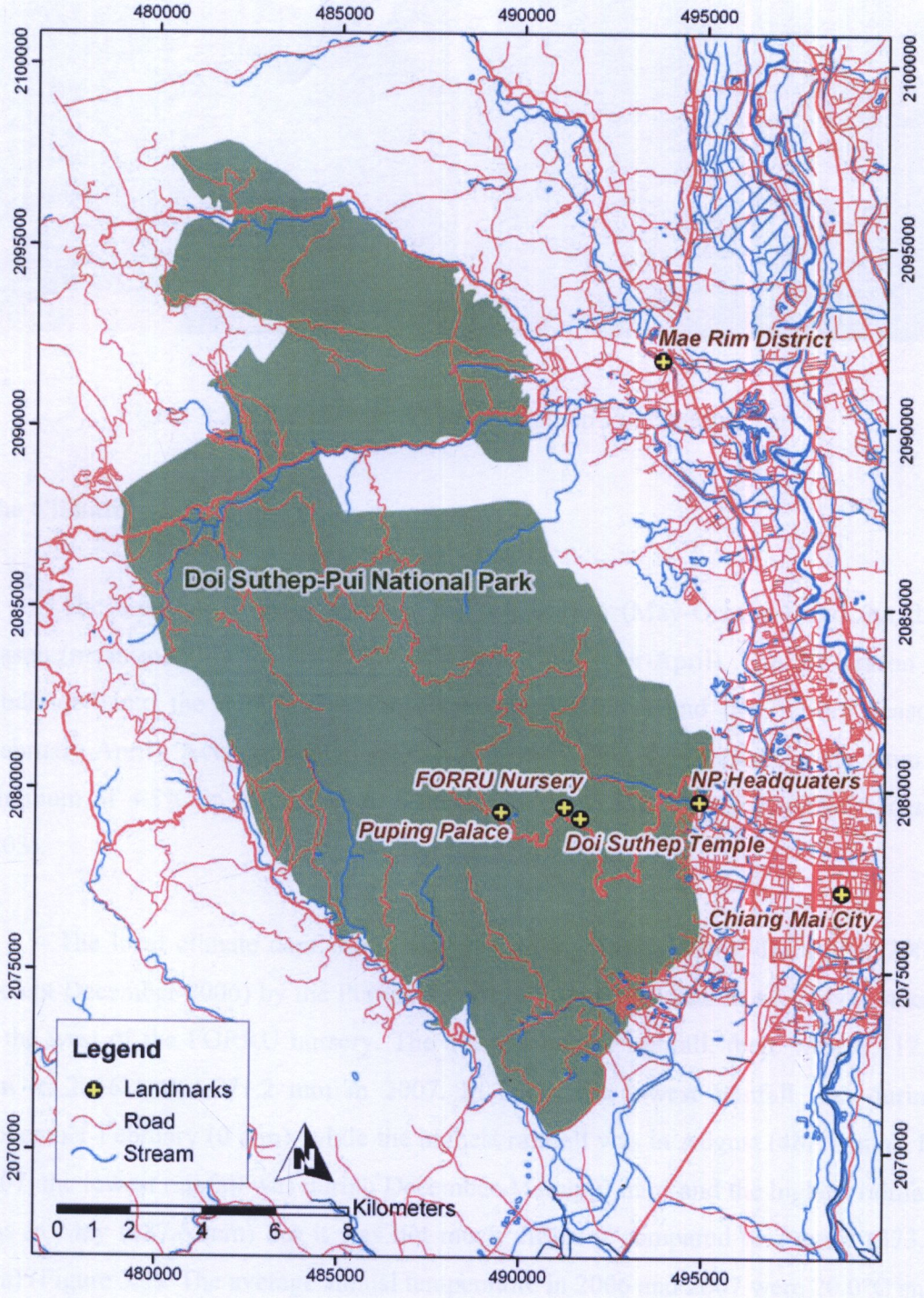

Figure 3.1 Doi Suthep-Pui National Park, Chiang Mai Province, Thailand. Green color shows Doi Suthep-Pui Nationl Park area and location of FORRU nursery (World Agroforestry Centre, 2009).

Figure 3.2 FORRU nursery in Doi Suthep-Pui National Park

The Climate

The area has 2 main seasons: the wet season (May-October) and the dry season (mean monthly rainfall below 100 mm, November-April). The dry season is subdivided into the cool-dry season (November-January) and the hot-dry season (February-April). Average annual rainfall is 2,094.9 mm. Temperatures vary from a minimum of 4.5°C in December to a maximum of 35.5°C in March (Elliott *et al.*, 2003).

The local climate data were measured during January 2006-December 2007 (except December 2006) by the Puping Palace, at elevation 1,350 m.a.s.l. about 4 km to the west of the FORRU nursery. The average annual rainfall, there were 2,112.9 mm in 2006 and 1,771.2 mm in 2007. In 2006, the lowest rainfall was during November-February (0 mm), while the highest rainfall was in August (483.1 mm). In 2007, the lowest rainfall was during December-March (0 mm) and the highest rainfall was in May (387.6 mm) but it was not much different compared to August (373.9 mm) (Figure 3.3). The average annual temperature in 2006 and 2007 were 20.0°C and 21.3°C, respectively. Each average annual minimum temperature was 15.6°C in 2006 and 16.7°C in 2007. Each average annual maximum temperature was 24.4°C in 2006 and 25.8°C in 2007 (Figure 3.4). The average annual relative humidity in 2006 and 2007 were 82.6% and 86.2%, respectively. The highest average monthly relative

humidity appeared in September, there were 92.9% in 2006 and 92.1% in 2007. The lowest average monthly relative humidity appeared in March, there were 61.9% in 2006 and 45.6% in 2007 (Figure 3.5).

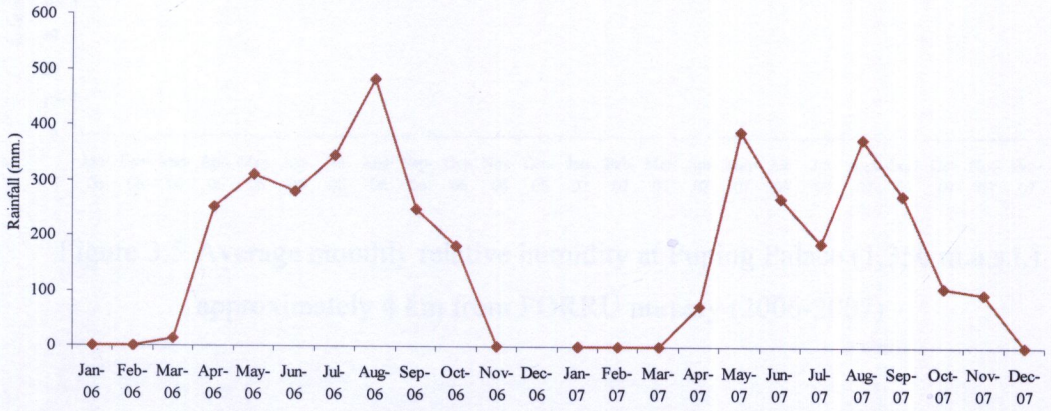

Figure 3.3 Average monthly rainfall at Puping Palace (1,350 m.a.s.l.) approximately 4 km from FORRU nursery (2006-2007)

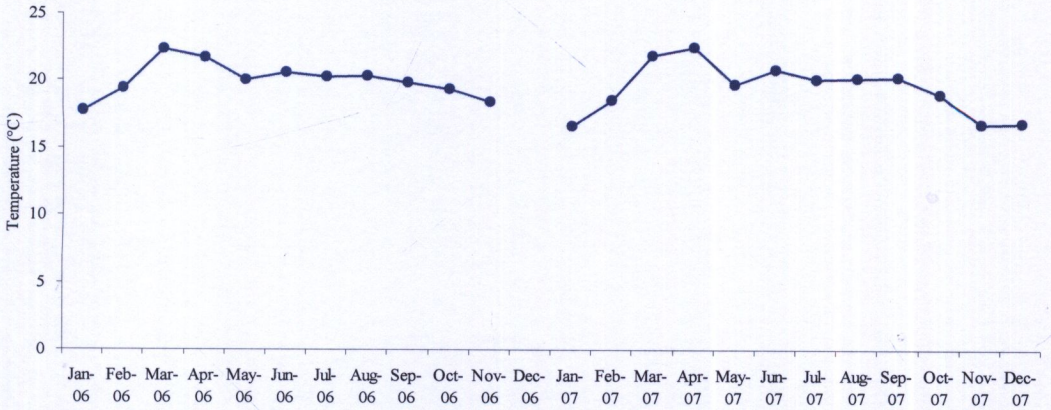

Figure 3.4 Average monthly temperature at Puping Palace (1,350 m.a.s.l.) approximately 4 km from FORRU nursery (2006-2007)

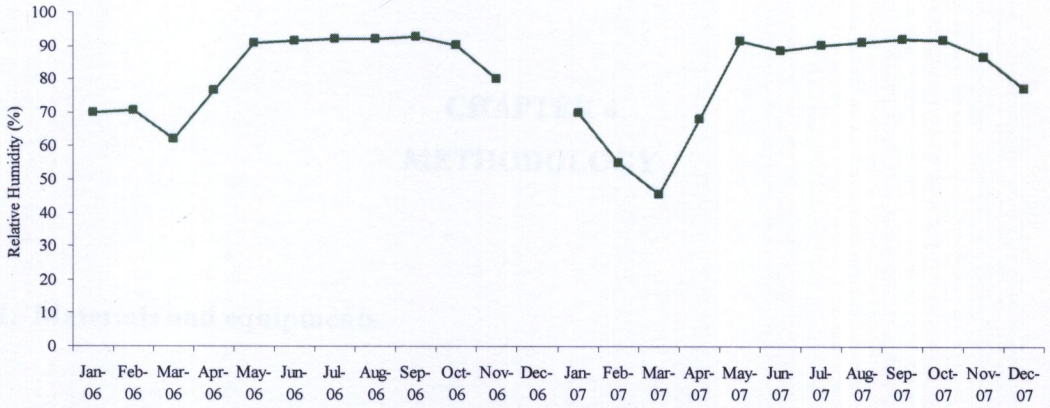

Figure 3.5 Average monthly relative humidity at Puping Palace (1,350 m.a.s.l.) approximately 4 km from FORRU nursery (2006-2007)

CHAPTER 4
METHODOLOGY

1. Materials and equipments

1.1 Chemical

- Indolebutyric acid (IBA) ($C_{12}H_{13}NO_2$)
- Naphthaleneacetic acid (NAA) ($C_{10}H_{12}O_2$)
- Seradix®, powder containing IBA 3,000 ppm
- Benomyl ($C_{14}H_{18}N_4O_3$)
- Captan ($C_9H_8Cl_3NO_2S$)
- Red lime
- 95% Ethanol
- Distilled water
- Osmocote, slow releasing fertilizer (14-14-14)

1.2 Rooting medium

- Coconut husk
- Forest soil
- Peanut husk
- Rice husk charcoal
- Sand
- Sawdust

1.3 Materials

- Secateurs
- Small black plastic bag (5.1 x 6.4 cm^2)
- Big black plastic bag (22.9 x 6.4 cm^2)

- Clear plastic bag (58.4 x 109.2 cm^2)
- Plastic basket (30.5 x 35.6 x 10.2 cm^3)
- Glove
- Mask
- Label sheet
- Rubber band
- Rope

1.4 Equipments for data collection

- Vernier caliper
- Ruler
- Data sheet
- Pencil
- Digital photo camera

2. Method

2.1 Species selection

Nine tree species, which rare or threatened with extirpation from northern Thailand and could not previously be grown from seeds according to the information stored in the CMU Herbarium Database (2007), were selected for propagation by cuttings (see Appendix for general characteristics of each species). The nine tree species were:

1. *Crypteronia paniculata* Bl. var. *paniculata* (Figure 4.1)
2. *Diospyros coaetanea* Flet. (Figure 4.2)
3. *Gardenia sootepensis* Hutch. (Figure 4.3)
4. *Haldina cordifolia* (Roxb.) Rids. (Figure 4.4)
5. *Ilex umbellulata* (Wall.) Loesn. (Figure 4.5)
6. *Mesua ferrea* L. (Figure 4.6)

7. *Rothmania sootepensis* (Craib) Brem. (Figure 4.7)
8. *Schoutenia glomerata* King ssp. *peregrine* (Craib) Roekm. & Hart. (Figure 4.8)
9. *Scleropyrum pentandrum* (Dennst.) Mabb. (Figure 4.9)

Figure 4.1 *Crypteronia paniculata* Bl. var. *paniculata* (Crypteroniaceae)

Figure 4.2 *Diospyros coaetanea* Flet. (Ebenaceae)

Figure 4.3 *Gardenia sootepensis* Hutch. (Rubiaceae)

Figure 4.4 *Haldina cordifolia* (Roxb.) Rids. (Rubiaceae)

Figure 4.5 *Ilex umbellulata* (Wall.) Loesn. (Aquifoliaceae)

Figure 4.6 *Mesua ferrea* L. (Guttiferae)

Figure 4.7 *Rothmania sootepensis* (Craib) Brem. (Rubiaceae)

Figure 4.8 *Schoutenia glomerata* King ssp. *peregrine* (Craib) Roekm. & Hart. (Tiliaceae)

Figure 4.9 *Scleropyrum pentandrum* (Dennst.) Mabb. (Santalaceae)

2.2 Stem collection

Medium-sized twigs or vigorous juvenile stem, which retained its greenish color, had well developed leaves and smooth bark (Khun and Dick, 1996), were surveyed and randomly selected as a source of cuttings from many mother trees for genetic diversity. Cuttings were collected in the morning by using a sharp and clean pair of secateurs, placed in clean plastic bags with a little water, and taken immediately to FORRU nursery, where they were watered thoroughly before propagation.

2.3 Experimental design

2.3.1 Experiment 1: Effect of auxin treatments

2.3.1.1 Cutting preparation

At FORRU nursery, moderately vigorous juvenile stems of all nine species were cut into heel shape and 10-20 cm long with node and leaf on the cuttings. The woody part and the fragile apical section were cut away. The length of cuttings or number of nodes varies from each species. For some species, if each node had a leaf or a bud, single node was used. In the other hand, for some species with short internode and lacking leaves or buds, the cuttings were included 2-3 nodes. Leaves on the cuttings were trimmed transversely by about 30-50% depending on leaf area to reduce water loss through transpiration. Big leaves species, the leaf was trimmed transversely to half or two thirds of its size, but for medium and small leaves species, the leaves were trimmed to one thirds or half of its size. The bases of the cuttings were cut into heel shape just below the node and then put immediately into water. Moreover, to prevent fungal infection, the prepared cuttings were immersed in solution of the fungicide Benomyl (0.3 g/1,000 cm^3) for 10 minutes.

2.3.1.2 Auxin preparation

Two commonly used of artificial auxins, IBA (BDH Laboratory supplies, Poole, England) and NAA (Acros, New Jersey, USA) were selected as rooting hormone for this study. Different concentrations and formulations of IBA were used as treatments. Seradix® (Yip In Tsoi & Jacks Ltd., Bangkok, Thailand), a rooting powder containing IBA 3,000 ppm, was selected. In addition, the mix of IBA and NAA was tested. The concentrations of IBA tested were 3,000, 5,000, and 8,000 ppm (3.0, 5.0, and 8.0 g/1,000 cm^3). The concentration of NAA tested was 2,500 ppm (2.5 g/1,000 cm^3). The auxin solutions were prepared individually by dissolving IBA or NAA in 95% ethanol then following by distilled water in a 1:1 ratio. Moreover, for combination IBA and NAA treatment, both auxins were mixed together before using in the ratio of 2:1 or 5,000:2,500 ppm. Thus, five treatments were obtained as below:

Treatment 1: Control (no auxin treatment)
Treatment 2: Seradix®
Treatment 3: IBA 3,000 ppm
Treatment 4: IBA 8,000 ppm
Treatment 5: IBA:NAA 5,000:2,500 ppm (or 2:1)

Leafy stem cuttings of all nine species were treated with rooting hormone treatments after immersed in fungicidal solution. For solution treatments, the base 5-10 mm of each cutting was dipped into the testing solution for 10 minutes. For powder treatment (Seradix®), caution was taken not to apply too much powder to the base of the cutting which can sometimes stop outgrowth of new roots. Therefore, quick drip and only a single layer with powder hormone were applied to the cutting base.

2.3.1.3 Propagator system

The low-technology, non-mist propagator system has proved successful in the propagation of many tree species (Kantarli, 1993; Khun and Dick, 1996; Ofori *et al.*, 1996; Mesén *et al.*, 1997; Negash, 2003; Vongkamjan, 2003; Subiakto *et al.*, 2005).

Big clear plastic bags of 58.4 cm diameter with a depth of 109.2 cm were used as propagators in this study. After applied on different rooting hormone treatments to the base of each cutting, the cuttings were then inserted in the rooting medium in small black plastic bags (5.1 x 6.4 cm^2). The rooting medium consisted of sand and rice husk charcoal mixed in the ratio of 1:1 which was prepared and watered one day prior to the date of preparation setting. A vertical hole about the same diameter as each cutting and 3-5 cm deep was made with pencil. The preset holes minimized the disturbance of the hormone while placing the cutting into the rooting medium. Cuttings were placed into the pre-made holes and the medium was made firm around by finger pressing and watering. Twelve cuttings were stored in a big clear plastic bag (58.4 x 109.2 cm^2). The plastic propagation bags were added about 1,000 cm^3 of water to each bag when originally prepared and tied closed which created circulating condensation and high humidity inside (Vongkamjan, 2003). Moreover, plastic bags were bounded firmly with bamboo stick holding to the beam of the nursery in order to prevent moisture loss. Each plastic propagation bag was labeled with the species of tree, the date of preparation setting, the treatment, and the number of the replicate. Cuttings were shaded in the nursery under black shade netting (approximately 30% sunlight) (Figure 4.10). The bags were checked weekly for tears and water level. Dead cuttings and dried leaves were removed from the bags to prevent diseases.

Figure 4.10 Propagator system

2.3.1.4 Statistical method

As described earlier, the experiment tested five treatments on each species. There were five replications of each treatment, with 12 cuttings for each replication. Therefore, a total of 300 leafy stem cuttings (12 cuttings x 5 treatments x 5 replicates) was tested and arranged in Complete Randomized Design (CRD) of each tree species.

2.3.2 Experiment 2: Effect of cutting source (position in stem)

Three parts, leafy stem cutting of *Rothmania sootepensis* were taken sequentially down the stem and their leaf areas trimmed as same as described in cutting preparation in Experiment 1. Three parts named as below:

Treatment 1: Top part
Treatment 2: Middle part
Treatment 3: Base part

Details of auxin preparation, propagator system, and statistical method in this experiment as same as Experiment 1, except number of auxin treatment and number of replications. Three auxin treatments were used in this experiment, including control, Seradix®, and IBA 8,000 ppm, since these treatments produced high number of cuttings with new shoots resulted from a preliminary determining in Experiment 1. Moreover, number of replication per treatment was decreased from five to three due to cutting source constraint.

2.3.3 Experiment 3: Effect of fungicides

Cuttings of *Ilex umbellulata* were obtained as described above and powder auxin was applied as the rooting hormone. Three common uses of fungicide were used as treatments, therefore, there were four treatments as follows:

Treatment 1: Control (no fungicide)
Treatment 2: Benomyl
Treatment 3: Captan
Treatment 4: Red lime

Benomyl and Captane were prepared in solution form before applying to the cuttings. The fungicide solutions were prepared by dissolving Benomyl (0.3 g/1,000 cm^3) or Captan (2 g/1,000 cm^3) in water. For the solutions, prepared cuttings were immersed in the solutions for 10 minutes before application of Seradix®. For the red lime treatment, cuttings were treated with Seradix® before coating with red lime at each cutting base.

In this experiment, plastic baskets (30.5 x 35.6 x 10.2 cm^3) were used instead on the plastic bags (described above) as cutting container and filled with rooting medium (mixed of sand and rice husk charcoal, 1:1) the same as for Experiments 1 and 2 (Figure 4.11). For each treatment; there were three replicates combination in a CRD, with 15 cuttings in each replicate.

(a) (b)

Figure 4.11 Two types of cutting container:
(a) small black plastic bag (12 cuttings/replicate) in Experiments 1 and 2
(b) plastic basket (15 cuttings/replicate) in Experiment 3

2.3.4 Experiment 4: Effect of leaf area

Leafy stem cuttings of *Crypteronia paniculata* were tested with the following leaf area treatments:

Treatment 1: Leafless
Treatment 2: Half leaves (Half of its original size)
Treatment 3: Full leaves (Whole leaf area)

All cuttings were treated with Benomyl solution as detailed in Experiment 1. Then, cuttings were treated with rooting hormone (Seradix®), inserted in the cutting container (plastic basket), and arranged according to the statistical method as explained in Experiment 3.

2.3.5 Experiment 5: Effect of rooting medium

Cuttings of *Mesua ferrea* were prepared as described in Experimental 1 to test with four different rooting medium treatments:

Treatment 1: Sand
Treatment 2: Sawdust
Treatment 3: A mixture of sand and rice husk charcoal (1:1 in volume)
Treatment 4: A mixture of sand, rice husk charcoal, and coconut husk (1:1:1 in volume)

All prepared cuttings were treated with Seradix® on the cutting base before being placed into different rooting media and arranged according to the statistical method described for Experiment 3.

3. Data collection

Cuttings were moved out of clear plastic bags and transplanted into black plastic bags (22.9 x 6.4 cm^2) after roots could be seen. The potting mixture consisted of forest soil, peanut husk, and coconut husk mixed in the ratio of 2:1:1. Potted plants were shaded in the nursery under black shade netting (approximately 30% sunlight) for two weeks. Then, the plants were moved out of the nursery and placed under full sunlight. Ten grams of "Osmocote" slow-release fertilizer (14-14-14) was placed on the media surface every three months.

The number of cuttings survivals, rooting, new shoots, and rooting with new shoots, together with numbers of roots and shoots, including lengths of roots and shoots were recorded when rooting appeared visibly. Moreover, the number of surviving plants, together with height and the root collar diameter (using vernier calipers) of plants from all treatments were measured every 30 days to determine effects of potting after removal from clear plastic bags.

4. Data analysis

4.1 Effects of auxin treatments, leaf area, fungicides, rooting medium, and sources of cutting (positions in stem)

Data collected of each treatment on each species when roots became visible were calculated as percentages and the mean number of cuttings surviving, rooting, shooting, and rooting with shooting, together with mean numbers and lengths of roots and shoots per cutting. These results were analyzed to test for significant differences among the treatments for each species by ANOVA (one-way analysis of variance), followed by least significant difference (LSD) tests if differences were detected at $P < 0.05$. Moreover, Relative Performance Score (RPS) and Comparison Among Species (CPS) were calculated for comparing within species and among species, respectively. Both indices had a maximum value of 100 and a minimum possible value of 0 (Vongkamjan, 2003).

Percentage of cuttings surviving, rooting, shooting, and rooting with shooting

$$\text{Percent (\% of cuttings per treatment)} = \frac{\text{SC}}{\text{TC}} \times 100$$

where: SC = number of cuttings surviving, rooting, shooting, or rooting with shooting

TC = total number of cuttings

Mean number of cuttings surviving, rooting, shooting, and rooting with shooting

$$\text{Mean (number of cuttings per treatment)} = \frac{\sum_{i=1}^{r} S_i}{r}$$

where: S_i = number of cuttings surviving, rooting, shooting, or rooting with shooting in replication i

r = total number of replications

Mean number of roots or shoots

$$\text{Mean (number of roots or shoots per cutting)} = \frac{\sum_{i=1}^{r} R_i}{r}$$

where: R_i = number of roots or shoots per cutting surviving with roots or shoots in replication i

r = total number of replications

Mean length of roots or shoots

$$\text{Mean length (cm of roots or shoots per cutting)} = \frac{\sum_{i=1}^{r} L_i}{r}$$

where: L_i = length of roots or shoots per cutting surviving with roots or shoots in replication i

r = total number of replications that cutting surviving with roots or shoots

Relative Performance Score (RPS)

A relative performance score was devised that combined both survival and vigour. The single variable, percentage of cuttings survival that produced both roots and shoots was represented to qualify survival, because cuttings that failed to produce shoots and roots would not ultimately contribute towards the production of planting stock. While, the other four variables, the mean numbers of both shoots and roots produced, including their mean lengths at the end of the experiments were represented to qualify vigour. This index was calculated by the following formula (Vongkamjan, 2003):

$$RPS = 50 \times \left[\frac{TrtS}{MaxS} + \left\{ \left(\frac{TrtNR}{MaxNR} + \frac{TrtNS}{MaxNS} + \frac{TrtRL}{MaxRL} + \frac{TrtSL}{MaxSL} \right) \times 0.25 \right\} \right]$$

where: Trt = the mean value for each individual treatment
Max = largest mean value among treatment
S = percentage of cuttings surviving with roots and shoots
NR = mean number of roots
NS = mean number of shoots
RL = mean root length
SL = mean shoot length

Comparison Among Species (CPS)

Comparison among species was more complex and problematic because each species was tested at a different time. Moreover, each species lasted for differing lengths of times due to major differences in the rapidity with which cuttings of different species rooted and grew. However, it was possible to devise an index that can be used to compare general "ease of cutting propagation" among species, based on the speed with which it was possible to produce a crop of cuttings ready for potting by selecting the best treatment for each species, then the data from that treatment were used to compare among species. Therefore, this index was devised that combined

from three components: ease of cutting propagation, survival, and vigour, by the following formula (Vongkamjan, 2003):

$$CPS = 33.33 \times \left[\frac{MinimumND}{SpeciesND} + \left\{ \frac{SpS}{MaxSpS} + \left[\left(\frac{NoR}{MaxNoR} + \frac{NoS}{MaxNoS} + \frac{RL}{MaxRL} + \frac{SL}{MaxSL} \right) \times 0.25 \right] \right\} \right]$$

where: ND = number of days from planting to termination of experiment and potting cuttings

Max = largest mean value among species

SpS = percentage of cuttings surviving with roots and shoots

NoR = mean number of roots

NoS = mean number of shoots

RL = mean root length

SL = mean shoot length

4.2 Effects of potting

Percentages of survival plants were evaluated. Moreover, to determine average relative growth rates, data on height and the root collar diameter of plants were calculated the relative growth rate of root collar diameter (RRGR) and relative growth rate of height (RHGR).

Survival percentage

$$Survival(\%) = \frac{SN}{TN} \times 100$$

where: SN = number of survived plants

TN = total number of plants

Relative growth rate of root collar diameter (RRGR)

$$\text{RRGR (\% increase per year)} = \frac{[\ln(\text{RCD2}) - \ln(\text{RCD1})]}{T} \times 365 \times 100$$

where: RCD1 = root collar diameter of plants in the first monitoring
RCD2 = root collar diameter of plants in the last monitoring
T = number of days between first and last monitoring
ln = natural log

Relative growth rate of height (RHGR)

$$\text{RHGR (\% increase per year)} = \frac{[\ln(\text{H2}) - \ln(\text{H1})]}{T} \times 365 \times 100$$

where: H1 = height of plants in the first monitoring
H2 = height of plants in the last monitoring
T = number of days between first and last monitoring
ln = natural log

CHAPTER 5
RESULTS

1. Water analysis

The water used in this study was accessible at the FORRU's nursery. It is the natural water flowing down from summit of Doi Pui through Huai Kok Ma and manageable by pipeline system to the nursery. The results of water analysis show that the water quality was suitable for this study (Table 5.1). According to surface water quality standard values (Type 3 for agricultural purposes) in National Environmental Quality Act B.E. 1992 (Pollution Control Department, 2010), that pH was in range 5-9 and heavy metals as Cu was lower than 0.1 ppm and Mn and Zn were lower than 1.0 ppm. Furthermore, total soluble salts, which are combinations of such cations as sodium, calcium, and magnesium with such anions as sulfate and chloride, were lower than 1,400 ppm (Hartmann *et al.*, 1990).

Table 5.1 Results of water analysis based on a single sample

Analysis of	**Result**
pH	6.80
Ammonium-Nitrogen (NH_4^+-N)	1.17 ppm
Calcium (Ca)	4.73 ppm
Chloride (Cl^-)	0.72 ppm
Magnesium (Mg)	0.46 ppm
Potassium (K)	1.60 ppm
Sodium (Na)	4.39 ppm
Sulfate (So_4^{2-})	1.56 ppm
Total Phosphorus (P)	0.01 ppm

Table 5.1 (continued)

Analysis of	Result
Copper (Cu)	ND
Manganese (Mn)	0.01 ppm
Zinc (Zn)	0.005 ppm

Remark: 1) Collecting date: 12 November 2009

2) Analysis laboratory: Central Laboratory, Department of Soil Sciences, Faculty of Agriculture, Chiang Mai University, Chiang Mai, Thailand

3) ND = non detectable (< 0.003 ppm)

2. Environmental conditions

Environmental conditions played an important role in the study for both propagation systems i.e. in and out plastic bags. Every aspect was measured thoroughly in this experimental study (Figure 5.1). The plastic propagator created higher relative humidity inside; over 85%, while the air temperature at FORRU's nursery ranged from 21-26°C. Moreover, the air temperature in plastic bag propagator was mostly higher than in the rooting medium, which ranged from 22-28°C and 21-24°C, respectively. For successful rooting of leafy cuttings, it has been suggested that a high relative humidity (more than 80%) must be maintained around cuttings (Vongkamjan, 2003), daytime air temperature should be 21-27°C (Samanond, 1983; Hartmann *et al.*, 1990; Na Nongkhai, 1999), and medium temperature should be 25-32°C (Hartmann *et al.*, 1990; Vaddhanaphuti, 1999).

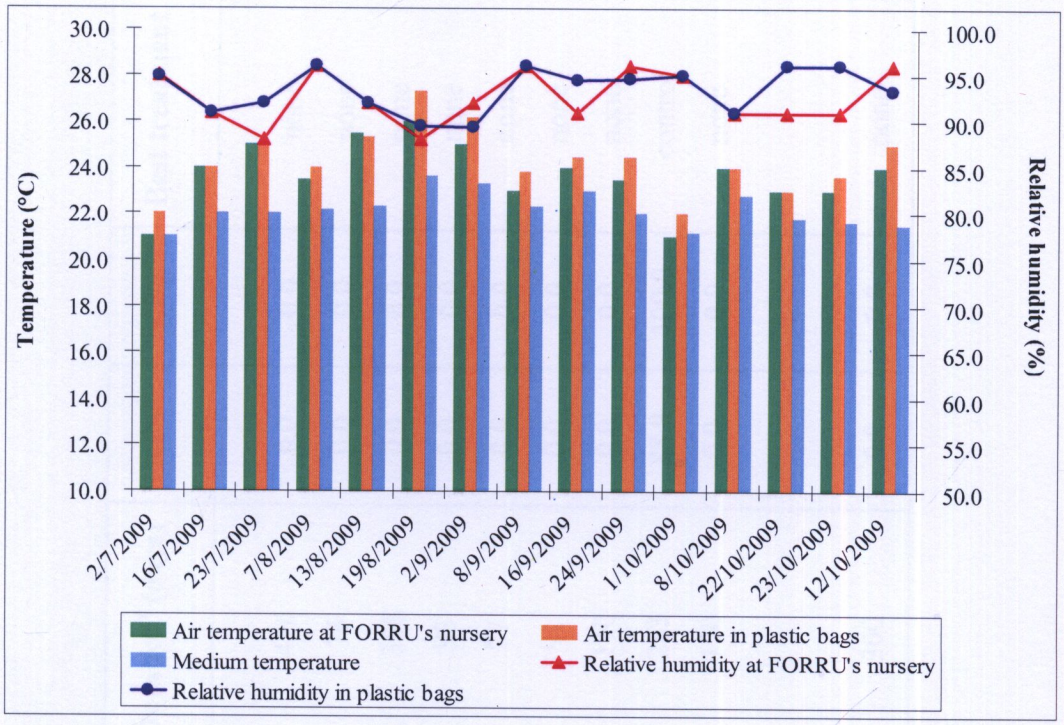

Figure 5.1 Average air temperature, average medium temperature, and average relative humidity at FORRU's nursery and in propagator system (n = 21 bags)

3. Vegetative propagation

Results of the species tested are shown in Table 5.2. ANOVA was used to detect the significance of the effects of each treatment. If differences were detected at $P < 0.05$, then least significant difference (LSD) was calculated. Performances scores of survival and vigour of each species were calculated, together with relative performance scores (RPS) and comparison among species (CPS).

Table 5.2 Results of cutting collection and performance

Species	Cutting collection date[a]	Experiment stopped date[b]	Duration[c] (days)	RPS[d]	CPS[e]	Best treatment
Experiment 1: Effect of auxin treatments						
Crypteronia paniculata	12/9/2008	12/11/2009	426	0.0	0.0	none
Diospyros coaetanea	22/5/2009	6/8/2009	76	0.0	0.0	none
Gardenia sootepensis	19/11/2008	12/11/2009	358	0.0	0.0	none
Haldina cordifolia	15/9/2008	17/12/2008	93	0.0	0.0	none
Ilex umbellulata	2/9/2008	18/11/2008	77	0.0	0.0	none
Mesua ferrea	20/5/2009	20/8/2009	91	0.0	0.0	none
Rothmania sootepensis	30/8/2008	18/12/2008	110	0.0	0.0	none
Schoutenia glomerata	9/9/2008	19/6/2009	283	88.9	100.0	control
Scleropyrum pentandrum	26/5/2009	12/11/2009	170	0.0	0.0	none
Experiment 2: Effect of cutting source (position in stem)						
Rothmania sootepensis	16/1/2009	12/11/2009	300	0.0	0.0	none

Table 5.2 (continued)

Species	Cutting collection date[a]	Experiment stopped date[b]	Duration[c] (days)	RPS[d]	CPS[e]	Best treatment
Experiment 3: Effect of fungicides						
Ilex umbellulata	31/7/2009	30/11/2009	122	0.0	0.0	none
Experiment 4: Effect of leaf area						
Crypteronia paniculata	30/7/2009	30/11/2009	123	0.0	0.0	none
Experiment 5: Effect of rooting medium						
Mesua ferrea	31/7/2009	30/11/2009	122	0.0	0.0	none

[a] Date cutting collection

[b] Cutting ready for potting

[c] Duration of experiment

[d] Relative performance score

[e] Comparison among species

none = no best treatment because there was no cutting survived with strong root for potting

3.1 Unsuccessful species on root development

Diospyros coaetanea, *Haldina cordifolia*, *Ilex umbellulata*, *Mesua ferrea*, and *Rothmania sootepensis* in Experiment 1 could not be propagated by the cutting technique and with the treatments applied. Although all species produced some new shoots, the cuttings died later without producing any roots. Fungal diseases caused some of the mortality. Three types of fungi could be distinguished growing on the *Ilex umbellulata* cuttings: a white mycelium, orange spot, and white spot (Figure 5.2).

(a) (b) (c)

Figure 5.2 Died cuttings of *Ilex umbellulata* with (a) white mycelium, (b) orange spot, and (c) white spot

3.2 Incomplete species due to time constraint

Due to time constraint of this study, some experiments had to end after four months: *Crypteronia paniculata* (Figure 5.3) and *Scleropyrum pentandrum* (Figure 5.4) in Experiment 1, *Rothmania sootepensis* (Figure 5.5), *Ilex umbellulata* (Figure 5.6), *Crypteronia paniculata* (Figure 5.7), and *Mesua ferrea* (Figure 5.8) in Experiments 2, 3, 4, and 5, respectively.

Some cuttings could survive with and without producing shoots. However, the number of live cuttings with new shoots decreased continually through the experiments and there was no root development.

Figure 5.3 *Crypteronia paniculata* after 426 days

Figure 5.4 *Scleropyrum pentandrum* after 170 days

Figure 5.5 *Rothmania sootepensis* after 300 days

Figure 5.6 *Ilex umbellulata* after 122 days

Figure 5.7 *Crypteronia paniculata* after 123 days

Figure 5.8 *Mesua ferrea* after 122 days

3.3 Species with minimal root development

Gardenia sootepensis in Experiment 1 produced new shoots in all treatments tested after 50 days but the number of cuttings with new shoots decreased throughout the experiment. Four cuttings; three in control treatment and one with IBA 3,000 ppm could survive after 358 days but only one cutting in the control treatment produced a single root (Figure 5.9). The root was short and was not strong enough for further potting.

Figure 5.9 Cuttings of *Gardenia sootepensis* in control treatment with new shoots and roots after 358 days

3.4 Species which root development

Only one species, *Schoutenia glomerata* in Experiment 1 produced roots. This species could be potted after 283 days from collection of leafy stem cuttings (Figure 5.10). Chemical treatments had no significant effect on the success of cutting propagation of this species (Table 5.3). Without chemical treatments, a highest of 8.3% of cuttings survived with both roots and shoots. Moreover, the control produced the highest mean number of cuttings surviving with shoots. With regard to vigour, chemicals produced no significant affects for all four variable. Calculation of the

relative performance score ranked the control as the most effective treatment (Table 5.4).

Figure 5.10 Cuttings of *Shoutenia glomerata* after 283 days

In comparison with other species, *Shoutenia glomerata* responded well to cutting propagation. Even though, roots developed very slowly. Evidently, only this species could produce roots from all five experiments. Consequently, this species had the maximum score of CPS (Table 5.2).

Table 5.3 Cutting propagation results of *Shoutenia glomerata*

Treatment	Survival				Rooting				With shoot				Roots+Shoots				No. of roots			No. of shoots			Root length			Shoot length		
	%[1]	Mean[2]	SD[3]	LSD[4]	%[5]	Mean[6]	SD[3]	LSD[4]	%[7]	Mean[8]	SD[3]	LSD[4]	%[9]	Mean[10]	SD[3]	LSD[4]	Mean[11]	SD[3]	LSD[4]	Mean[12]	SD[3]	LSD[4]	Mean[13]	SD[3]	LSD[4]	Mean[14]	SD[3]	LSD[4]
Control	11.7	1.4	1.7	ns	8.3	1.0	1.0	ns	11.7	1.4	1.7	ns	8.3	1.0	1.0	ns	1.9	2.0	ns	0.7	0.7	ns	5.2	2.2	ns	0.9	0.1	ns
Seradix®	8.3	1.0	0.0	ns	8.3	1.0	0.0	ns	5.0	0.6	0.6	ns	5.0	0.6	0.6	ns	2.8	1.9	ns	0.8	0.8	ns	8.4	3.0	ns	1.1	0.4	ns
IBA 3000 ppm	15.0	1.8	0.8	ns	11.7	1.4	1.1	ns	5.0	0.6	0.9	ns	5.0	0.6	0.9	ns	3.2	2.4	ns	0.8	1.1	ns	4.5	1.2	ns	0.6	0.2	ns
IBA 8000 ppm	6.7	0.8	0.8	ns	5.0	0.6	0.9	ns	0.0	0.0	0.0	ns	0.0	0.0	0.0	ns	1.0	1.7	ns	0.0	0.0	ns	7.8	2.5	ns	0.0	0.0	ns
IBA:NAA 5000:2500 ppm	15.0	1.8	1.3	ns	11.7	1.4	1.1	ns	5.0	0.6	0.6	ns	5.0	0.6	0.6	ns	2.8	1.6	ns	0.4	0.6	ns	8.6	1.7	ns	1.0	0.0	ns

[1] % of cuttings surviving

[2] mean number of cuttings surviving

[3] standard deviation

[4] least significant different $P = 0.05$ (ns = no significant differences among all treatment)

[5] % of cutting surviving with roots

[6] mean number of cuttings surviving with roots

[7] % of cutting surviving with shoots

[8] mean number of cuttings surviving with shoots

[9] % of cutting surviving with roots and shoots

[10] mean number of cuttings surviving with root and shoots

[11] mean number of roots per cutting

[12] mean number of shoots per cutting

[13] mean length of root per cutting (cm)

[14] mean length of shoot per cutting (cm)

Table 5.4 Relative performance score of *Shoutenia glomerata*

Treatment	Cuttings surviving with roots and shoots		Vigour					Total[8]
	%[1]	Survival score[2]	No roots[3]	No shoots[4]	Root length[5]	Shoot length[6]	Vigour score[7]	
Control	8.3	50.0	1.0	1.4	5.2	0.9	38.9	88.9
Seradix®	5.0	30.0	1.0	0.6	8.4	1.1	39.0	69.0
IBA 3,000 ppm	5.0	30.0	1.4	0.6	4.5	0.6	31.6	61.6
IBA 8,000 ppm	0.0	0.0	0.6	0.0	7.8	0.0	16.6	16.6
IBA:NAA 5,000:2,500 ppm	5.0	30.0	1.4	0.6	8.6	1.0	41.9	71.9

[1] % of cuttings surviving with roots and shoots

[2] calculated of survival score

[3] mean number of roots per cutting

[4] mean number of shoots per cutting

[5] mean length of root per cutting (cm)

[6] mean length of shoot per cutting (cm)

[7] calculated of vigour score

[8] total performance score

4. Plant growth

Percentages survival, relative growth rate of the root collar diameter (RRGR) and relative growth rate of height (RHGR) were calculated for *Shoutenia glomerata* plants, since only this species produced strong roots development for potting. All 34 plants were measured every month for survival, height, and the root collar diameter from 2 July 2009-29 November 2009 (5 months or 150 days).

4.1 Survival percentage

The number of surviving plants continually decreased from the 1st month to the 5th month (Figure 5.11). By 5th month, only 20 plants (58.8%) remained alive.

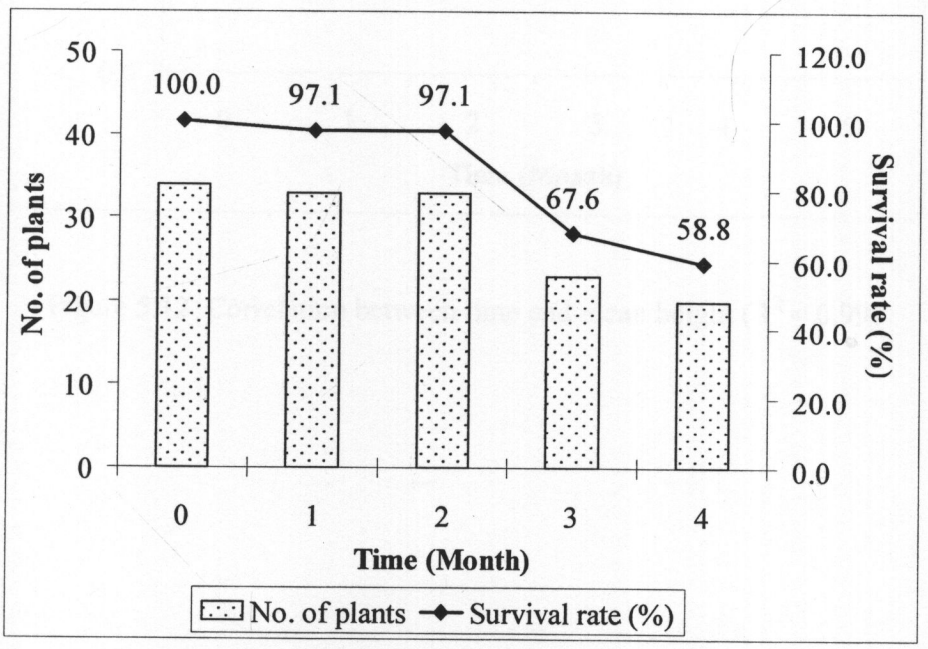

Figure 5.11 Number of plants and survival percentage rate of *Shoutenia glomerata*

4.2 Relative growth rate (RGR)

RRGR (cm) of *Shoutenia glomerata* plants was 17.3 %/year and RHGR (cm) was 73.6 %/year. Figure 5.12 and 5.13 show steady growth of the plants over time.

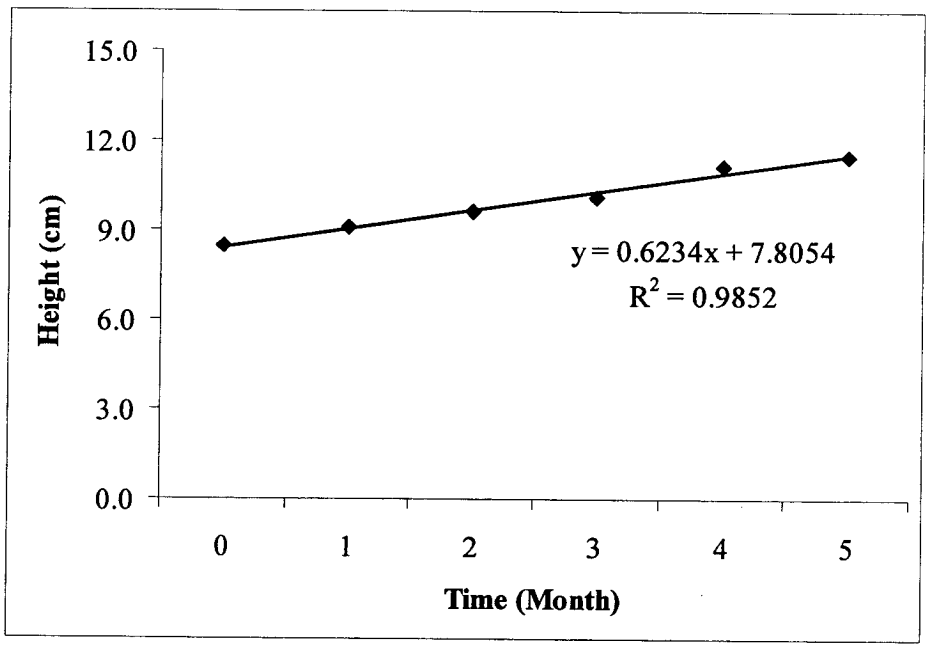

Figure 5.12 Correlation between time and mean height ($R^2 = 0.99$)

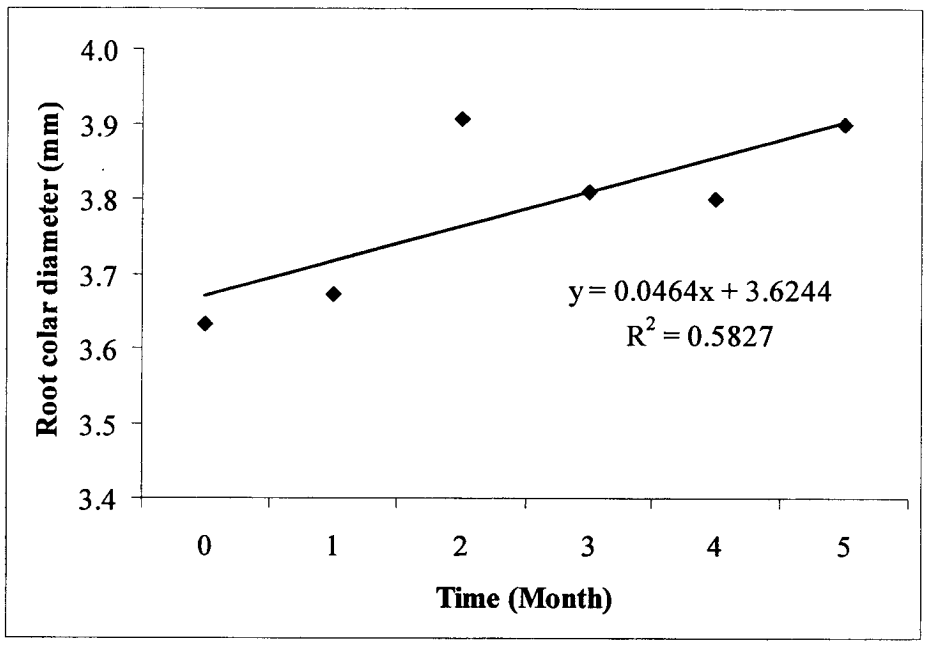

Figure 5.13 Correlation between time and mean root colar diameter ($R^2 = 0.58$)

CHAPTER 6
DISCUSSION

1. Vegetative propagation

Nine rare tree species which had not been successfully propagated from seeds, due to unpredictable fruiting seasons, irregular fruiting, recalcitrance of the seeds, and attack by insects and other animals were selected for experiments on cutting propagation. Rooting of cuttings was achieved only with *Gardenia sootepensis and Shoutenia glomerata* and that was achieved without any hormone treatment (although several hormone treatments were tested).

Successful rooting without applied auxin has been reported of cuttings of several tropical tree species, such as *Allanblackia floribunda* (Atangana *et al.*, 2006), *Milicia excelsa* (Ofori *et al.*, 1996), *Nauclea diderrichii* (Leakey, 1990), and *Trema orientalis* (Vongkamjan, 2003). For *Gardenia sootepensis and Shoutenia glomerata*, rooting occurred very slowly (358 and 283 days, respectively). Low numbers of cuttings survived with both roots and shoots (0.3 and 8.3%, respectively).

Applying the results obtained during the study reported here, the production on 100 *Shoutenia glomerata* trees for planting would require preparation of 723 leafy stem cuttings (assuming 8.3% survival with both roots and shoots). Other treatments should be tried to accelerate rooting and increase survival of cuttings with both roots and shoots.

With the methods used in this study, none of the other seven species tested with different treatments (application of rooting hormones, fungicides, leaf areas manipulation, rooting media, and cutting positions on the source plant) produced roots.

Therefore, these species could not be propagated from cuttings. The cuttings developed brown leaves, rapidly wilted and followed by stem collapse. Cuttings must retain leaves for successful root initiation and development (Ofori *et al.*, 1996; Shiembo *et al.*, 1996; Tarragó *et al.*, 2005), for supply of auxins and nutritional factors (Jarvis, 1986; Gaspar *et al.*, 1997). Moreover, the selection of juvenile material (Negash, 2002; Kibbler *et al.*, 2004) is critical factors for the success of cuttings propagation. Therefore, further experiments should be carried out to test other propagation methods to produce planting stock of rare tree species.

2. Propagation system

Maintenance of appropriate environmental conditions (Kantarli, 1993; Atangana *et al.*, 2006) is critical for the success of cuttings propagation. Cool air and high humidity at the leaf surfaces minimize water loss from the material, while a moist, warm rooting medium encourages fast root development (Matthews, 1999). Root initiation in cuttings is temperature-driven, but high air temperatures tend to promote bud elongation in advance of root initiation, and to increase water loss from the leaves (Hartmann *et al.*, 2002). Therefore, it has been suggested that the air temperature should be lower than medium temperature because if air temperature is not controlled or is too high, most of the stored food in cutting stems would be rapidly utilized for shoot development and thus root development would be hampered (Wright, 1973).

In contrast with this study, the rooting medium was always cooler than the air surrounding the cuttings. To maintain high air humidity in the propagator system, some water was added at the base of the plastic bags to avoid water stress in the cuttings. This may result in low temperature in rooting medium. Moreover, during weekly checking, it was necessary to shake the plastic bags to make them transparent. Therefore, some water fell to the bottom of the plastic bags, possibly resulting in low temperature in rooting medium.

3. Relative growth rate of *Shoutenia glomerata*

During tree production to restore forest, all trees must reach a plantable size (about 50 cm high) in the planting season (at the beginning of the rainy season, mid June to mid July, in northern Thailand). Also, it is not efficient to keep trees in nursery more than one year. However, from the results, it is not be possible to produce viable planting stock of *Shoutenia glomerata* in less than one year, because it needs 283 days from the collected day of leafy stem cuttings until transferring the rooted cuttings into pots. Moreover, relative growth rate was very slow. Potted plants would have to be kept further in the nursery for 67.7 months or at least five years before they would be ready for planting. I would like to suggest that further research for this species is needed, especially in terms of time consuming and applying fertilizer utility.

4. Cutting container

In addition to the fact that small black plastic bags used for container of cuttings was not suitable to store in clear plastic bag propagator. They can be easily damaged by falling down when moved by taking out of some dead leaves, adding some water, and observing rooting. Thus, plastic basket can provide a more effective cutting container because it helps to solve this problem. Moreover, plastic baskets contained less rooting medium and consumed less preparing time compared with small black plastic bags.

CHAPTER 7
CONCLUSIONS AND RECOMMENDATIONS

Conclusions

1. Vegetative propagation by cuttings was not suitable for the rare tree species tested in this study owing to time-consuming in rooting production and difficulty in achieving successful cuttings that survived with both roots and shoots as the percentage of survival was very low. As a result, it is not possible to mass produce quality planting materials for forest restoration and for species conservation.

2. Various treatments for cuttings, including auxin applications, fungicides, leaf areas, rooting media, and sources of cuttings (position in the stem) did not stimulate root formation of rare tree species.

3. Root production and the relative growth rate of potted plants were very slow. Therefore, it was not possible to produce viable planting stock within one year.

4. It was obvious that using plastic baskets as cutting containers are more appropriate than using black plastic bags.

5. Using clear plastic bags as a simple non-mist propagator system was not productive for cutting propagation of rare tree species.

Recommendations

1. Experiments should be carried out to identify optimum environmental conditions and test other propagation systems with well drainage to remove excess water, such as making drainage holes around the rooting bed or setting automatic sprinkler system.

2. Further methods are needed to propagate rare tree species from seed, as some species might be grown from seed with different seed preparation of each tree species before germination, for example, *Gardenia sootepensis* (73.3% germination by soaking in water 12 hours), *Mesua ferrea* (20.0% germination by scarification), *Rothmania sootepensis* (83.9% germination by soaking in water 36 hours), and *Scleropyrum pentandrum* (21.7% germination by scarification) (Koonyodying, unpublished).

3. Use their wildings by transplanting from forest to the nursery at the beginning of rainy season. Moreover, for conservation purposes, tentative wildings should be dug up within a distance of 5 m around its parent tree while the optimum height of potential wildings for transferring should not be more than 20 cm (Kuarak, 2002).

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APPENDIX

General characteristics and seedling descriptions of the selected nine tree species in Doi Suthep-Pui National Park (Gardner *et al.*, 2000; Maxwell, 2001; BIOTIK, 2010; Kuaraksa *et al.*, unpublished)

1. ***Crypteronia paniculata* Bl. var. *paniculata***

Common name (Thai): กะอาม, ขี้มด

Family: Crypteroniaceae

Habit:	Evergreen tree
Habitat:	Degraded teak & bamboo + deciduous forest; mixed evergreen + deciduous, seasonal forest; evergreen forest with pine
Elevation range:	620-1,650 m.a.s.l.
Flower month:	December-January
Fruit month:	February-March
Leaf month:	January-December
Bark:	Thick, dark grey-brown, closely ridged and fissured, usually flaking into thin strips, inner bark pale brown, inner bark pale brown, fibrous, without latex or sap
Leaf:	8-17 x 4-7 cm, young leaves bluish-purple at first, turning pinking-brown, mature leaves mid-green, completely smooth or finely hairy below, twigs dark red-brown
Flower:	< 0.5 cm, cream or pale yellow-green, male and female on different trees, branched spike-like clusters towards end of wigs
Fruit:	0.2-0.5 cm, dark purple, globose or ovoid, often faintly grooved, with persistent calyx and style, often finely hairy, dry, splitting into 2 sections, many tiny seeds with papery wing
Abundance:	Down to a few individual, in danger of extirpation

Stage of problem why it rare in nature: Pollination/reproductive (no embryo)

Appendix (continued)

2. *Diospyros coaetanea* Flet.

Common name (Thai): ลำบิด, ลำตาควาย

Family: Ebenaceae

Habit:	Evergreen or deciduous tree
Habitat:	Degraded teak & bamboo + deciduous forest
Elevation range:	400-500 m.a.s.l.
Flower month:	May-June
Fruit month:	July-October
Leaf month:	April-February
Bark:	Dark grey, deeply fissured, inner bark pinkish-orange
Leaf:	To 20 x 7 cm, elliptic or oblong, blunt or slightly pointed at both ends, mature leaves hairy below, ± 20 pairs of ± parallel side veins
Male flower:	Smooth stalks 2-3 cm, calyx bell-shaped, divided 1/2 into 4 (5) lobes, blackish hair on both sides, corolla tubular divided 1/5, hairy outside, smooth inside, 10-14 stamens, smooth
Fruit:	2-3 cm, greenish-yellow, globose or oval, woody, skin > 2 mm thick, smooth or slightly hairy near base
Abundance:	Down to a few individual, in danger of extirpation
Stage of problem why it rare in nature:	Fruiting time and seed dormancy (germinated late rainy)

Appendix (continued)

3. ***Gardenia sootepensis* Hutch.**

Common name (Thai): คำมอกหลวง

Family: Rubiaceae

Habit:	Deciduous tree
Habitat:	Deciduous dipterocarp-oak forest; degraded teak & bamboo + deciduous forest; mixed evergreen + deciduous, seasonal forest
Elevation range:	375-900 m.a.s.l.
Flower month:	April-May
Fruit month:	July-September
Leaf month:	May-March
Bark:	Pale cream or grey, quite smooth, peeling in thin plates, no thorns
Leaf:	9-28 x 4-15 cm, oblong or obovate with blunt tip and rounded base, young leaves pale orange, silvery hairy, mature leaves glossy dark green above, finely hair below, leaf buds broadly conical covered with sticky yellow resin
Flower:	Large, pale green or white turning rich yellow-orange, solitary at end of twigs or on stout stumps in leaf axils
Fruit:	3-5 cm, bright green, oval with distinct nipple at top and 5 shallow ridges, fleshy with many small seeds
Abundance:	Medium
Stage of problem why it rare in nature:	Natural regeneration (animals break seed coat)

Appendix (continued)

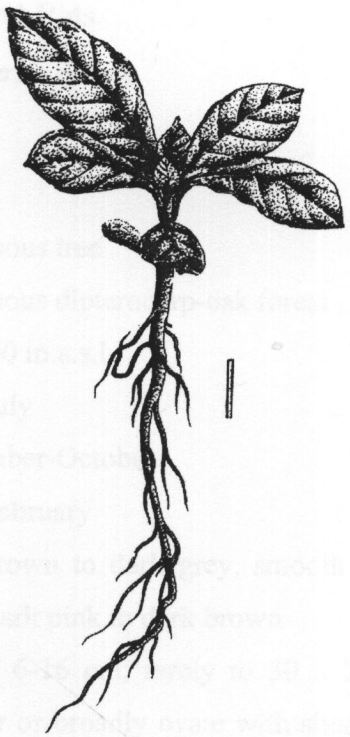

Figure A1 *Gardenia sootepensis* seedling after160 days
(Illustrator: Damrongchai Saengkam)

Appendix (continued)

4. ***Haldina cordifolia*** **(Roxb.) Rids.**

Common name (Thai): ขว้าว, ตุ้มควาย

Family: Rubiaceae

Habit:	Deciduous tree
Habitat:	Deciduous dipterocarp-oak forest
Elevation range:	500-500 m.a.s.l.
Flower month:	June-July
Fruit month:	September-October
Leaf month:	May-February
Bark:	Pale brown to dark grey, smooth or scaly & finely fissured, inner bark pink to dark brown
Leaf:	8-20 x 6-16 cm, rarely to 30 x 20 cm, opposite in 2 rows, circular or broadly ovate with short tip and heart-shaped base, young leaves pale green with pink stalks, mature leaves thin with scattered rough hairs above and denser soft hairs below
Flower:	< 1.0 cm, pale yellow or pinkish, slightly fragrant, in dense spherical heads
Fruit:	0.3-0.4 cm in globose heads, dry with hard partitions between the seeds, each fruit splitting from top sections with a persistent central axis and calyx, tiny seeds pointed at one end, narrowly winged
Abundance:	Down to a few individual, in danger of extirpation

Stage of problem why it rare in nature: Pollination/reproductive (no embryo)

Appendix (continued)

5. ***Ilex umbellulata*** **(Wall.) Loesn.**

Common name (Thai): เน่าใน, ไคร้มด

Family: Aquifoliaceae

Habit:	Evergreen tree
Habitat:	Mixed evergreen + deciduous, seasonal forest; primary evergreen forest
Elevation range:	500-1,350 m.a.s.l.
Flower month:	April
Fruit month:	July-October
Leaf month:	January-December
Bark:	Pale cream or grey-brown, thin, finely cracked, inner bark brown with pale streaks
Leaf:	5-15 x 3-6 cm, simple, alternate, spiral, oblong or elliptic, blunt or slightly pointed at both ends, mature leaves completely smooth dark green and glossy above
Flower:	White or pale green, regular, in dense head-like clusters at upper leaf axils or behind leaves
Fruit:	0.6-0.8 cm, pale green turning bright red with yellow flesh, globose or ovoid with 4-8 grooves, persistent calyx at base, 4-8 hard stones
Abundance:	Medium

Stage of problem why it rare in nature: Pollination/reproductive (no embryo)

Appendix (continued)

6. ***Mesua ferrea* L.**

Common name (Thai): บุนนาค

Family: Guttiferae

Habit:	Evergreen tree
Habitat:	Mixed evergreen + deciduous, seasonal forest; primary evergreen forest
Elevation range:	725-725 m.a.s.l.
Flower month:	May-June
Fruit month:	August-September
Leaf month:	January-December
Bark:	Dark brown, flaking, inner bark with very sparse pale yellow latex
Leaf:	5-13 x 1.2-4 cm, simple, opposite, narrowly elliptic or lanceolate, tapering at both ends, young leaves pale pink, covering the whole tree for just a few days each year, mature leaves dark green above, pale grey (glaucous) below, side veins very numerous but extremely faint
Flower:	5-7.5 cm, white, bisexual, solitary or paired in leaf axils, hanging face downwards
Fruit:	2.5-3.5 cm, dark orange or purple-brown, ovoid with pointed tip, not splitting, densely covered with woody fibres, often with drops of resin, base enclosed in fleshy sepals, 1-4 hard dark brown seeds
Abundance:	Rare
Stage of problem why it rare in nature:	Natural regeneration (seed predation and diseases)

Appendix (continued)

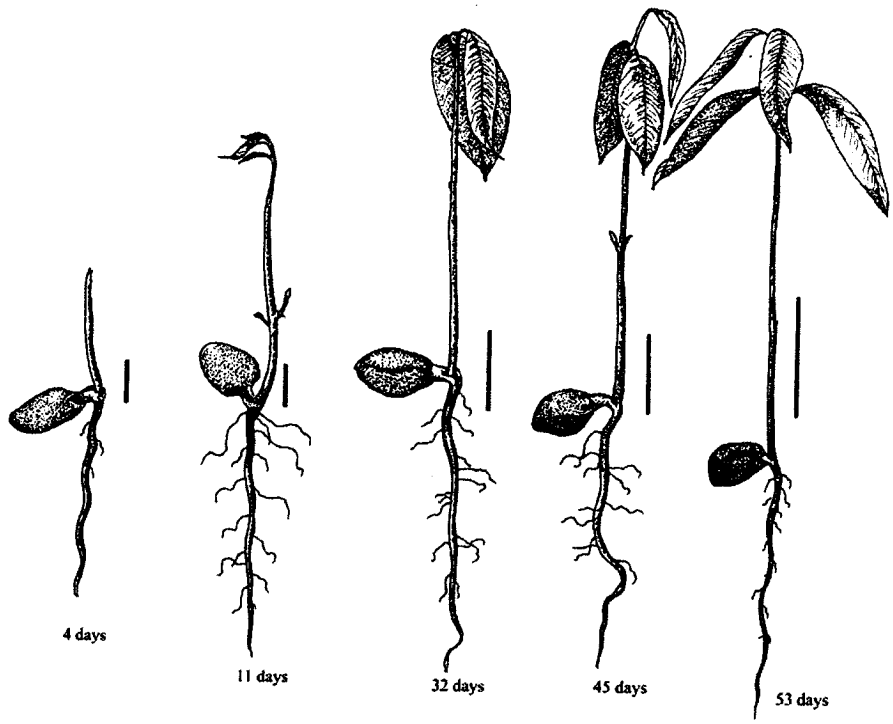

Figure A2 Various stages of *Mesua ferrea* seedling
(Source: FORRU)

Appendix (continued)

7. ***Rothmania sootepensis* (Craib) Brem.**

Common name (Thai): สแหล่งหอมไก่

Family: Rubiaceae

Habit:	Evergreen tree
Habitat:	Mixed evergreen + deciduous, seasonal forest; primary evergreen forest
Elevation range:	450-1,250 m.a.s.l.
Flower month:	February-March
Fruit month:	September-June
Leaf month:	January-December
Bark:	Red-brown or dark grey-brown, finely cracked and flaking
Leaf:	8-14 x 2-5 cm, opposite, often planar, narrowly elliptic, pointed or tapering at both ends, base slightly asymmetric, mature leaves completely smooth, dull dark green above, pale green with sunken glands (1 mm) in vein axils below, 6-8 pairs of arched side veins
Flower:	5.0-7.5 cm, white with red-purple dots near mouth inside, in cluster of 1-5 flowers on a short common stalk at end of leaves
Fruit:	2.5-6.0 cm, dark yellow-brown, ellipsoid or subglobose, slightly sunken at the top with a short point in the middle, divided into 2 chambers each with several flattened seeds surrounded by a slimy orange pulp
Abundance:	Medium

Stage of problem why it rare in nature: Pollination/reproductive (few in fruits)

Appendix (continued)

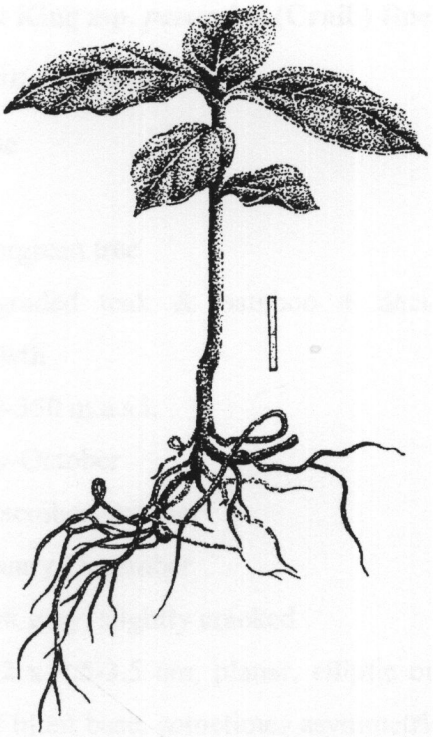

Figure A3 *Rothmania sootepensis* seedling after 218 days
(Illustrator: Damrongchai Saengkam)

Appendix (continued)

8. *Schoutenia glomerata* King ssp. *peregrine* (Craib) Roekm. & Hart.

Common name (Thai): รวงผึ้ง, สายน้ำผึ้ง

Family: Aquifoliaceae

Habit:	Evergreen tree
Habitat:	Degraded teak & bamboo + deciduous forest; secondary growth
Elevation range:	350-350 m.a.s.l.
Flower month:	July-October
Fruit month:	September-October
Leaf month:	January-December
Bark:	Dark grey, slightly cracked
Leaf:	4-12 x 1.5-3.5 cm, planar, elliptic or oblong with pointed tip and blunt base, sometimes asymmetric, no teeth, mature leaves rather thick, dark green and glossy above, paler with tiny creamy-brown star-shaped hairs which easily rub off below
Flower:	± 1.3 cm, bright yellow, bisexual, in short unbranched clusters at leaf axils, to 3 cm, sometimes densely flowered and head-like
Fruit:	0.5-1.0 cm, globose with enlarged calyx at base, hairy, dry and not splitting, 1.0-1.5 cm diameter
Abundance:	Down to a few individual, in danger of extirpation

Stage of problem why it rare in nature: Pollination/reproductive (few in fruits)

Appendix (continued)

9. *Scleropyrum pentandrum* (Dennst.) Mabb.

Common name (Thai): ขี้หนอน, เหมือดคน

Family: Santalaceae

Habit:	Evergreen tree
Habitat:	Generally found in disturbed habitats on sandy soil, as well found in semi and dry evergreen forests, in open forest near stream and in lowland dipterocarp forest
Elevation range:	500-1,000 m.a.s.l.
Flower month:	January-March
Fruit month:	August-October
Bark:	Bole straight with fascicle of sharp spines on trunk or branches, bark deeply fissured while juvenile stem striate, light brown, inner bark light brown-cream
Leaf:	Leaves simple alternate and spiral, 10- 20 x 2.5-9 cm, narrowly to broadly elliptic or ovate, apex acute, shortly acuminate, base attenuate, margin entire, blade leathered to coriaceous, upper surface shining, glabrous, under surface glabrescent along midrib, midrib canaliculated above, primary vein single, secondary veins oblique to the midrib, widely parallel and anastomosing at margin, tertiary veins obscure, petiole pubescent, stipules absent
Flower:	Flowers small, grouped in fascicle of catkins, cauliflorous, unisexual, male flower smelling unpleasant, shortly pedicelled while female flower sessile
Fruit:	Drupe with pear shape or ovoid, 1.8-3.5 by 1.3-2.6 cm, stalked, green turning yellow when ripen

Appendix (continued)

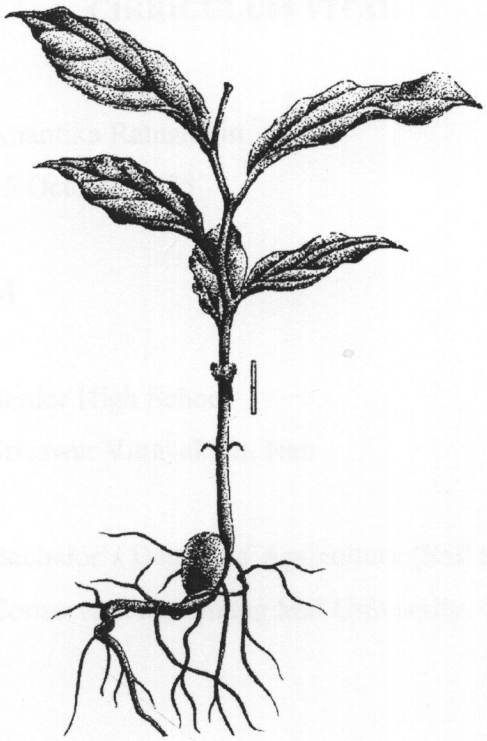

Figure A4 *Scleropyrum pentandrum* seedling after 361 days
(Illustrator: Damrongchai Saengkam)

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2) Centre for Environmental Health, Toxicology and Management of Chemicals (ETM)