

THE EFFECT OF ENVIRONMENTAL FACTORS ON SEED  
GERMINATION, AND SEEDLING GROWTH AND  
SURVIVAL OF CASTANOPSIS INDICA

MISS SANSANEE SRIAMONMONGKOL

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The effect of environmental factors on seed germination, and seedling growth and  
survival of *Castanopsis indica*

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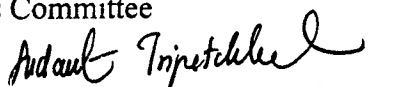
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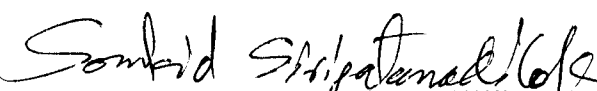
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#### Abstract

The branches of *Castanopsis indica* trees are a highly suitable substrate for mushroom cultivation. This has encouraged local villagers in northern and northeastern Thailand to cut *C. indica* and other similar species trees extensively, which may threaten these populations. Understanding effects of environmental factors on *C. indica* seed germination and seedling establishment are important for the management of this and other similar species. Three experiments were conducted to examine seed germination in the field and seedling growth in the nursery. First, the effects of canopy and ground vegetation cover on seed germination was conducted in two community forests and one national park, in Amphur Nahaeo, Loei province. In each study area, 1x1 m<sup>2</sup> plots were selected on the basis of different levels of canopy and ground vegetation cover. Second, the effects of predation on seed germination and seed mortality were examined, using the same methods as in the previous experiment, but half of the plots were covered with plastic nets or metal



cages to protect the seeds from predators. Third (the third experiment examined) the effects of light intensity and water availability on seedling growth in the nursery were examined. Planted seedlings in one of three light treatments (full sunlight, partial shade and heavy shade) and two watering treatments (low moisture and high moisture).

The results suggested that the effects of vegetation cover and canopy cover on seed germination were small. The main factor affecting seed germination was seed predation, which caused seed losses of up to 92.5%. The results also suggested that the metal cages were probably more effective in protecting seeds from predators than the plastic netting. The percentage of seed predation increased with increased vegetation cover and seed predation seemed to be slower in the less disturbed forest (the National Park) during the early germination period (October-January).

In the nursery all *C. indica* seedlings given full sunlight and only natural rainfall water died after 45 days, whereas partial shade (30% of full sunlight) and heavy shade (13% of full sunlight) increased survival to 60% and 33% respectively. Radial, vertical and leaf growth was significantly less in the forest (5% of full sunlight) when compared to the treatments in the nursery, but seedlings in the forest had 100% survival. Radial growth was also significantly greater in the partial shade treatment than under heavy shade.

In conclusion, in the field, predation appeared to be the main factor affecting seed germination, while in the nursery light intensity seemed to be the most important factor regulating seedling growth, and water availability seemed most important to seedling survival.

Keywords: *Castanopsis indica* / seed germination / seed growth / seed predator / seed mortality / light intensity / water availability



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### บทคัดย่อ

กิ่งของต้นก่อข้าว (*Castanopsis indica*) เป็นกิ่งไม้ที่มีความเหมาะสมในการใช้เป็นวัสดุสำหรับการเพาะเห็ดหอม ดังนั้นชาวบ้านในหมู่บ้านต่างๆ ที่ตั้งอยู่ในทางภาคเหนือ และภาคตะวันออกเฉียงเหนือของประเทศไทยจึงนิยมนำไม้ก่อข้าวและไม้ก่อชนิดอื่นๆ มาใช้ในการเพาะเห็ดหอมอย่างแพร่หลาย ซึ่งอาจทำให้เกิดปัญหาการลดลงและการสูญพันธุ์ของก่อข้าวในอนาคต การจัดการเพื่อให้มีการใช้ประโยชน์จากต้นก่อชนิดต่างๆ โดยเฉพาะก่อข้าว อย่างยั่งยืนนั้น จำเป็นต้องเข้าใจถึงปัจจัยทางสิ่งแวดล้อมที่มีผลต่อการงอก และการเจริญเติบโตของต้นก่อ ในการศึกษานี้ได้ทำการศึกษาผลของปัจจัยทางด้านสิ่งแวดล้อมต่อการงอกของเมล็ดในป่าซึ่งใช้เป็นพื้นที่ศึกษา และการเจริญเติบโตของต้นอ่อนในโรงเรือนเพาะชำ โดยแบ่งการศึกษาเป็น 3 ส่วนคือ การศึกษาแรกเป็นการศึกษาผลของปริมาณเรือนยอดปกคลุม และปริมาณพืชพรรณปกคลุมต่อการงอกของเมล็ดในพื้นที่ป่าชุมชน 2 แห่ง และป่าอุทยานนาเห้วแห่งชาติ 1 แห่ง (อำเภอนาเห้ว จังหวัดเลย) ทำการคัดเลือกแปลงศึกษาขนาด 1x1 ตารางเมตร ในแต่ละพื้นที่ศึกษา ภายได้เงื่อนไขของความแตกต่างกันของปริมาณเรือนยอด และปริมาณพืชพรรณปกคลุม การศึกษาที่สองเป็นการศึกษาผลของสัตว์กินเมล็ดเป็นอาหารต่อการงอก และการตาย (การสูญหายของเมล็ด) โดยวิธีการศึกษาเหมือนการศึกษาแรก และใช้ตาข่ายพลาสติก หรือกรงโลหะในการป้องกันความสูญหายของเมล็ดจากสัตว์ และการศึกษาที่สามเป็นการศึกษาผลของความเข้มแสงและการให้น้ำ ต่อการเจริญเติบโตของต้นก่อในโรงเรือนเพาะชำ ที่ความเข้มแสง 3 ระดับ (ได้รับแสงเต็มที่ ได้รับร่มเงาบางส่วน และ อยู่ในที่ร่ม) และการให้น้ำ 2 ระดับ (ได้รับน้ำเป็นประจำทุกวัน และ ได้รับน้ำฝนตามธรรมชาติ)

ผลการศึกษาพบว่า ปริมาณพืชพรรณปกคลุม และ เรือนยอดปกคลumnันมีผลต่อการงอกของเมล็ดเพียงเล็กน้อย แต่ปัจจัยหลักที่มีผลต่อการงอกของเมล็ดคือ สัตว์กินเมล็ดเป็นอาหารจำพวก กระรอก และหนู เมล็ดส่วนใหญ่ คือร้อยละ 92.5 ของเมล็ดที่ใช้ในการศึกษาทั้งหมดสูญหายไปก่อนที่จะเกิดการงอก นอกจากนี้ยังพบว่าปริมาณพืชพรรณปกคลุม และชนิดของวัสดุที่ใช้ในการป้องกันเมล็ดในการถูกทำลายโดยสัตว์นั้นมีผลต่อเปอร์เซ็นต์การเข้าทำลายโดยสัตว์ โดยพื้นที่ที่มีปริมาณพืชพรรณปกคลุมมากนั้นจะมีเปอร์เซ็นต์การเข้าทำลายเมล็ดโดยสัตว์สูง แนวโน้มการเข้าทำลายเมล็ดโดยสัตว์ในป่าที่ถูกรบกวนโดยกิจกรรมของมนุษย์น้อยกว่า (ป่าอุทยานนาแห้วแห่งชาติ) ช่วงการงอกเริ่มต้น (ตุลาคม-มกราคม) จะช้ากว่าในป่าชุมชน การใช้กรงโลหะสามารถป้องกันเมล็ดถูกกัดกิน หรือสูญหายโดยสัตว์ได้ดีกว่าตาข่ายพลาสติก

ในการศึกษาการเจริญเติบโตของต้นอ่อนในโรงเรือนเพาะชำนั้นพบว่า ต้นอ่อนที่ได้รับแสงแดดเต็มที่ (แสง 100%) แต่ไม่มีการให้น้ำนั้นจะตายหมดภายในเวลา 45 วัน ในขณะที่ต้นอ่อนที่ได้รับแสงบางส่วน (แสง 30%) และอยู่ในร่มเงา (แสง 13%) แต่ไม่มีการให้น้ำพบว่า มีเปอร์เซ็นต์การอยู่รอดเป็น 60 และ 33 เปอร์เซ็นต์เป็นลำดับ เมื่อเปรียบเทียบการเจริญเติบโต และการอยู่รอดของต้นอ่อนในป่า และในโรงเรือนเพาะชำพบว่า อัตราการเจริญเติบโตของต้นอ่อนในป่า (แสง 5%) ทั้งในทางด้านความสูง ขนาดลำต้น และพื้นที่ใบนั้น ต่ำกว่าอัตราการเจริญเติบโตของต้นอ่อนที่โรงเรือนเพาะชำ แต่ต้นอ่อนในป่านั้นมีการอยู่รอดทั้งหมด (ร้อยละ 100 ของต้นอ่อนทั้งหมด) แต่เมื่อเปรียบเทียบการเจริญเติบโตและการอยู่รอดของต้นอ่อนที่ปลูกในโรงเรือนเพาะชำภายใต้สถานะที่ได้รับน้ำ และปริมาณแสงต่างกันพบว่าอัตราการเจริญเติบโตทางด้านขนาดของลำต้นของต้นอ่อนที่ได้รับน้ำ และได้รับแสงบางส่วนนั้นสูงกว่าอัตราการเจริญเติบโตของต้นอ่อนที่ได้รับน้ำ และอยู่ในร่มเงา กล่าวโดยสรุปคือ ในภาคสนามนั้นปัจจัยสำคัญที่มีผลต่อการงอกของเมล็ดคือ การถูกทำลายโดยสัตว์ ในขณะที่ในโรงเรือนเพาะชำนั้นปริมาณของแสง เป็นปัจจัยสำคัญที่มีผลต่อการควบคุมการเจริญเติบโตของต้นอ่อน และระดับของการได้รับน้ำจะมีความสำคัญต่อการอยู่รอดของต้นกอข้าว

คำสำคัญ (Keywords) : ก่อข้าว (*Castanopsis indica*) / การงอกของเมล็ด / การเจริญเติบโตของต้นอ่อน / สัตว์ที่กินเมล็ดเป็นอาหาร / การตายของเมล็ด / แสง / น้ำ



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## Nomenclature

- RGR = Relative growth rate, %/year for vertically, radial and leaf area
- RPI = Relative performance Index
- Ln = A number showing the power to which a certain fixed number (the base) must be raised to yield a specified number
- $R^2$  =  $1 - (e'e)/(y'y)$  where  $e$  is the vector of residuals and  $y$  is the dependent variable measured in deviations from the mean
- P-value = The measured probability of a finding occurring, i.e. rejecting the null hypothesis, by chance alone given that the null hypothesis is actually true. By convention, a  $p$  value  $< 0.05$  is often considered significant. (There is less than a 5% probability that the finding)



## Chapter 1

### Introduction

#### 1.1 Background

Thailand's forest cover has been reduced from 53% [1] to about 22.8% or 111,010 km<sup>2</sup> [2] due to intensive forest invasion. The invasion of the forest causes both direct and indirect effects such as loss of wildlife habitat, erosion of watersheds and worsening rural poverty. Forests in rural of Thailand are resources for local villagers which supplement their living by hunting and collecting forest products for sale [3]. One forest product is *Castanopsis indica*. The branches of this tree are used for cultivating mushrooms. For example, villagers can earn 25,000-30,000 baht/year/village from mushroom cultivation [4]. Currently, villagers are interested in cultivating mushrooms in northern and northeastern Thailand, which encourages the villagers to cut more *C. indica* trees and branches and other similar species without thinking about the long-term consequences [5]. In addition to the effects of the loss of whole trees, branch cutting may also have other long-term effects. For example, a study conducted by Gupta and co-workers [6] found that branch cutting, apart from having effects on the production of the parent tree, can also change the structure of the tree canopy, which causes direct effects on the growth and survival of the seedlings under the parent tree by changing the light intensity and thereby changing the water availability, species composition and competition for available light and space. These changes may also effect seed germination [6]. Therefore this

study aims to examine the effects of light intensity and water availability on seed germination and seedling growth rate of *Castanopsis indica* both under natural conditions and under more controlled conditions. In doing so, I aim to understand the important factors that effect seedling survival, which can be used to germinate *Castanopsis indica* seedlings and plant them to replace the *Castanopsis indica* trees that have been cut. If seedlings can be effectively re-planted, this could provide for the sustainable use of *Castanopsis indica* and could serve as a model for the sustainable use of other forest products.

## 1.2 Objectives

1. Compare *Castanopsis indica* seed germination under different levels of canopy cover and ground vegetation cover in the field.
2. Study the effects of light intensity and water availability on *Castanopsis indica* seedling growth rate and survival in the nursery.

## 1.3 Scope of study

1. The study areas included the community forests of Ban Huay Nam Pak, Ban Bor Muang Noi, and Nahaeo National Park, Amphur Nahaeo, Loei province.
2. Study the relationship between ground vegetation cover, and canopy cover on seed germination and seed mortality.
3. To test the effects of light intensity and water availability on seedling growth and survival in the nursery.

#### 1.4 Expected benefits

1. To provide information for villagers and others interested in cultivating *C. indica* (and other related species) in nurseries (or in the field) from seeds or seedling.
2. To provide methods of maximizing germination in the field, and light and water requirements for optimal seedling growth in the nursery.

## CHAPTER 2

### Literature Review

#### 2.1 Castanopsis indica [6, 7, 8]

Thai indigenous name: Ko lim, Ko khao

Laos indigenous name: Ko ket

**Stem:** medium to large, evergreen tree species, 20-35 m in height when mature and 40 to 100 cm in diameter when grown under optimal conditions. It has a low branching pattern. The bark is brownish black, which is shallowly and longitudinally fissured.

**Leaves:** It has simple, oblanceolate leaves. The base of the leaf is oblique acuminate; the tip of the leaf is acuminate. The edges are serrated toward the tip of the leaf. The lower part is oily green while the upper part is greenish with a scattered covering of hair. The leaves are 10 to 25 cm long and 5 to 7 cm in width with 14 to 20 pairs of leaf-veins (figure 2.1).

**Fruits:** The fruits have a diameter of 0.6-1.3 cm and are 1.5-1.8 cm in height. The seed coat is brownish yellow, covered with brown hairs. The inner seed coat has fewer hairs. The seeds (nuts) are eatable and in Thailand develop between May and August and fall between September and November (figure 2.1).

**Cupule:** The cupule is round, 1-2 cm in diameter and 2-2.5 cm in height. The wall is 1-1.2 mm thick. The outer part of the wall has stellate hairs with sharp tips



0.5-1 cm long arranged in lines with 5 to 6 layers per line. Each line is 0.3-0.5 cm apart. The inner wall contains soft hairs that are yellowish white, which wrap the whole fruit. When the fruits mature, the wall is broken into 4 parts, which allows the fruit to be released from the wall.

Habitat: In Nahaeo National Park, *Castanopsis indica* can be found in hill evergreen forest and mixed deciduous forest 500 to 800 m above sea level [3].

Distribution : Laos, Vietnam, Cambodia, Thailand, Burma, India, China, Nepal and Taiwan

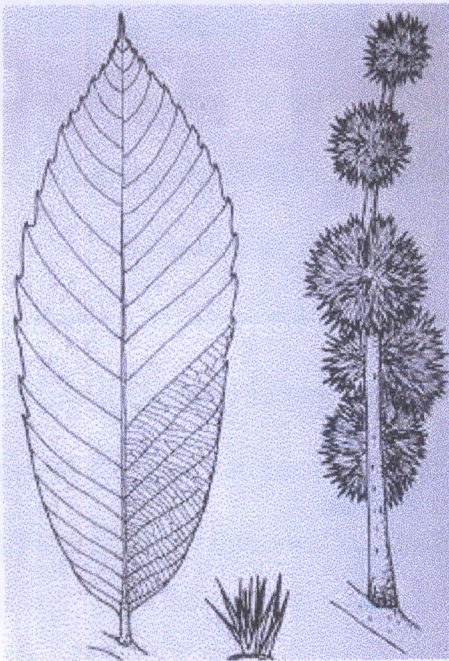


Figure 2.1 leaf and fruits of *Castanopsis indica* [8]

## 2.2 Structure of Seeds

A true seed is a fertilized mature ovule that contains an embryonic plant, stored food material (rarely missing), and a protective coat or coats. [The embryo is made up of one or more cotyledons, a plumule (embryonic bud), hypocotyl (stem portion), and a radicle (rudimentary root) [9].]

1. Embryo: The embryo is comprised of the embryonic axis and one or more cotyledons. The axis incorporates the embryonic root (radicle), the hypocotyl to which the cotyledons are attached, and the shoot apex with the first true leaves (plumule) [10].

2. Storage Tissues: Considerable variability exists among plant species in the sources of carbohydrates and other growth requirements for seed germination and early development of seedlings [10].

3. Seed coat (Testa): The testa is of considerable importance to the seed because it is often the only protective barrier between the embryo and the external environment. The protective nature of the seed coat can be ascribed to the presence of an outer and inner cuticle, often impregnated with waxes and fats, and one or more layers of thick-walled, protective cells [10].

## 2.3 Classification of seeds

Generally, seeds are often classified by their storage ability, as either recalcitrant or orthodox. **Recalcitrant** seeds have a higher moisture content (more than 6%) and have shorter dormancy and can be stored for only short periods and not at low temperatures. **Orthodox** seeds can be readily stored under low temperatures, and have a lower moisture content (4-6%) [11].



Seeds can also be organized by ecological characteristics. Seeds of pioneer species usually have rapid germination and short viability, can be readily stored under artificial conditions, have relatively low seed moisture and generally show various degrees of dormancy [11]. Seeds of non-pioneer species are usually difficult to store, but if collected fresh are easy to germinate and establish in nurseries, and germinate abundantly in natural forest [11] (see section 2.4 below for definitions of pioneer/ non-pioneer).

## 2.4 Factors affecting seed germination

In order for a seed to germinate, it must be placed in environmental conditions favorable to this process. Among the conditions required are adequate supplies of water, a suitable temperature and composition of gases in the atmosphere, (as well as light for certain seeds). Requirements for specific conditions varies with the species and variety and is determined both by the conditions that prevail during seed formation and even more by hereditary factors. Frequently it appears that there is some correlation between the environmental requirements for germination and the ecological conditions occurring in the habitat of the plant and the seeds [10]. These factors are considered in more detail below.

### 2.4.1 Effects of light on seed germination of pioneer and non-pioneer species

Among cultivated plants, light does not have much direct influence on seed germination. However, light has indirect effects on seed germination because light conditions can break seed dormancy in tropical forest tree seeds. This may

include changes in the red: far-red ratio (R:FR), temperature changes [12], fluctuating soil moisture [13] or some combination of these.

Gitay and Noble [14] proposed that tropical forest tree species could be divided into two guilds, pioneers and non-pioneers.

Pioneer species were distinguished by their dependence on canopy gaps for seed germination and seedling establishment. The implication was that pioneers (light demanders) had seed dormancy that could be broken by gap conditions (increased irradiance and red: far-red ratio (R:FD), elevated or fluctuating temperatures), associated with a requirement of their seedlings for high irradiance, and thus the guilds were naturally distinct. The hypothesis was based on circumstantial evidence that pioneer species were never found as young seedlings in forest shade, and only occurred as young plants in canopy gaps or in other disturbed areas such as road sides and farms. Various studies have shown that some pioneer species such as *Musanga cecropioides* and *Terminalia* sp. require light for germination (photoblasticity). Swaine & Whitmore [15] also had found that for pioneers, the seedlings required high irradiance. Thus, although pioneers species may germinate in deep shade, they will usually be overlooked because seedlings die swiftly under low irradiance. Pioneers were no more resistant to the effect of high irradiance than non-pioneers. Kyereh *et al.*'s [16] study of the effects of irradiance on seed germination found that irradiance probably has mostly indirect effects through the regulation of seed temperature, or more probably seed moisture content. They found that only 3 species of tropical forest trees, out of 17 species tested, was effected by low red: far-red ratios (R:FD) at low irradiance in a shadehouse. The effects of increasing canopy openness on seed germination does not appear to be due

to desiccation as it is not stronger in seeds with high water content, which can still be killed by relatively small losses of moisture [17].

Evidently photoblasticity is a characteristic of a subset of pioneers, and is associated with small-seeded, fleshy-fruited species that are found in the soil seed bank. This is a useful subdivision of the guild, but it would seem impractical to redefine pioneers on this basis because few species have been tested experimentally and because the existing definition is useful and widely applicable. It seems probable that the photoblastic group will be relatively small, and include particularly the small stature genera such as *Musanga*, some *Ficus*, *Cecropia*, *Trema*, and *Harungana* [11].

Hawthorne [19,20] described a subdivision of the more species-rich non-pioneer guild on the basis of a seedling's ability to survive and grow in deep forest shade. The non-pioneer tree species can be classified into:

1. non-pioneer light demanders (NPLD): have higher mortality, most dying before exceeding a meter or so in height when growing in deep shade.
2. non-pioneer shade bearers (NPSH): have lower mortality and continue to grow in deep shade, eventually becoming established trees.

Chloroplasts absorb light most strongly at approximately 675 nm and allow wavelengths longer than 720 nm to be transmitted completely. Because of this, sunlight passing through green leaves has a very low red/far-red ratio. The phytochrome photoequilibrium established by such light can be as low as 0.15, if the light has passed through a sufficient number of leaves [10]. Most light requiring, dormant seeds will not germinate under such conditions. Indeed, if they are exposed

to this light for many hours over several days they will probably be forced into secondary dormancy. They become dormant as a result of irradiation with canopy light and will germinate later only when a dormancy-releasing factor, such as chilling or light, has been experienced. Seeds of *Bidens pilosa*, for example, when forced into dormancy by canopy light, become light - requiring and subsequently germinate when exposed to light of suitable quality (relatively high photoequilibrium values) or direct sunlight [20]. This mechanism accounts for the paucity of seedlings on forest floors and the flush that follows the appearance of a gap in the leaf canopy, brought about when individual trees die and fall or when tree clearance occurs. Thus, the seed bank in forest soils may be rich but germination is held in check, to a large extent by the far-red environment.

As mentioned, the number of leaves through which light passes determines the spectral energy distribution of canopy light. Hence the effects on germination will often vary according to the density of leaf cover. No investigations on this have been carried out in field situations, but it has been simulated under experimental conditions using seeds of *Plantago major*. When placed under increasing leaf cover, provided by *Sinapis alba* plants, germination of these light-requiring seeds was increasingly inhibited as the cover became more dense (measured by the leaf area index) and the phytochrome photoequilibrium beneath the leaves decreased. Variations in the density of a leaf canopy occur in the field as buds break and leaves expand and later senesce and fall. Thus, light-sensitive seeds are exposed to changing light environments throughout the growing season, with concomitant effects on their dormancy breakage and germination. However, because other environmental factors,

such as temperature, are also influential, that even when shade ends, seed germination will not necessarily proceed [10].

The importance of the light environment in lowering mortality by pathogens has been confirmed experimentally by Holmes *et al.*, Bazzaz *et al.* and Denslow [20, 21, 22]. It was found that 17 of 18 species incurred pathogen-caused mortality. Such mortality was significantly lower for seedlings in sun than in shade. The high radiation, high temperature, and low humidity of tropical forest light-gaps have been shown to be inimical to fungal pathogens that cause damping-off of seedlings [23, 24,25].

#### 2.4.2 Water

Goo [26] studied the absorption process in *Pinus thenabergn*. He found that the absorption during seed germination can be divided into 3 steps.

The initial step, seeds absorb a lot of water because of imbibition pressure, in which water melts colloids inside the seeds throughout the capillary space and intercellular space. Colloids will expand and cause imbibition pressure, which can be as much as 100 atm in some species of seeds [27]. While the seeds are absorbing water they also release the heat. Mostly the absorption occurs through the seed coat where the seed coat is thinnest (near the micropylar). At the same time, the seeds also require gas exchange [28]. According to an experiment with *Picea abies* and *Pinus sylvestris*, moisture is 50 percent of seed mass, but the ability to absorb depends on the temperature [29], the higher temperature, the better the absorption.

Second stage is on day two and day three. The absorption in this stage is just a little faster than the first stage. Hear, the cells starts to divide.

The last stage, the seeds are able to absorb more and more rapidly, which is called the second rapid absorption period. Cells divide more than the first 2 stages so absorption also increases.

The absorption within each part of the seeds is not the same. In an experiment with *Abies procera* Rehd by Edward [31] concluded that the absorption of the seed coat may be obtained from the empty seed. Edward's [31] study of *Abies procera* seeds at 25 °C found that this absorption stopped by the third day of the experiment. Edward [31] also found that viable seeds have as much as 60% moisture in their seed coats. Whereas the moisture within the seed, which is used to evaluate total absorption when grown at low temperatures (5 °C) had better absorption than seeds that were grown at higher temperatures (25 °C). These result were similar to these result of Mayer and Poljakoff-Mayber [27].

The absorption depends on the protein content of the seeds as well. Seeds that have more protein, such as sunflower (*Helianthus sp.*) which are 25% protein, are able to absorb more water than seeds in the *Fagaceae*, which have on average only 3% protein [32]. Starches usually do not take part in this absorption. However, carbohydrates will swell where there is low pH and high temperatures, but this condition rarely occurs.

There have been few absorption experiments using seeds with thick seed coats. One of them, using *Fraxinus mandshurica* var, *Japonica*, suggested that it was difficult to specify the process of this absorption. Nevertheless, experiments with species of the *Fagaceae* family (*Quercus nuttallii*, *Q. palustris*, *Q. falcata* and *Q. rubra*) Bonner [33] found that the pericarp will help in absorption through the vascular opening. A separate pericarp also can help the absorption.

### 2.4.3 Temperature

Different seeds have different temperature ranges within which they germinate. At very low temperatures and very high temperatures the germination of all seeds is prevented. The precise sensitivity is very different among species. A rise in temperature does not necessarily cause an increase in either the rate of germination or in its percentage. Germination as a whole is therefore not characterized by a simple temperature coefficient [34].

Temperature is important in the breakdown of the hard waterproof coats present in seeds of many species such as *Antidesma bunius* and *Cleidon spiciflorum* [35]. Heat and fluctuating temperatures are especially important in hard seeds of tropical, subtropical, and Mediterranean climates. Heat is one treatment that can overcome seed coat dormancy and enhance germination [36].

Alternating temperatures breaks dormancy of many species in the field. A periodic alternation of temperature is required for germination of *Nicotiana tabacum*, *Holcus lanatus*, *Agrostis alba* and many others. The most usual cases of such alternations are diurnal ones, between a low and high temperature. An examination of some of the results obtained with alternating temperatures suggests that what determining germination is the actual alternation of temperature. The temperatures chosen, between which the seeds were alternated, appears to be of secondary importance, provided they are in a range within which the seeds can germinate and their viability is not affected. For example, 69% of *Agrostis alba* seeds germinated if alternated between 12 °C and 21 °C; 95% germinated with an alternation between 21 °C and 35 °C. At a constant 12 °C the germination was 49%, and it was 53% at 21 °C [34].



As far as buried seeds are concerned, it seems that vegetation cover is important, for it can influence the amplitude of the temperature fluctuation. This effect is seen rather well in an experiment in which the diurnal temperature fluctuation in soil under different sizes of gaps in the overlying vegetation was determined, together with the germination of buried *Holcus lanatus* seeds. Gap size clearly influenced the amplitude of the temperature fluctuation, which in turn appeared to correlate with the germination percentages. The temperature amplitude that a soil can achieve after vegetation clearance varies according to the depth at which the seed is measured. It has been suggested that the dampening of the amplitude can be used by dormant seeds to sense the depth at which they are buried. Clearance of vegetation can therefore have important effects, in addition to the light environment, on the top layer of the soil [10].

High solar radiation, which heats dry seeds, followed by low night temperatures can act to crack hard seed coats. Under some conditions, fire is equally effective in certain species [10]. Portlock *et al* [37] who studied effects of fire on seed germination, found that high temperatures (from fire) were able to induce seed germination of *Acacia sieberiana* and *A. gerrardii*. The observed pattern of increasing and then decreasing percentage germination suggested that a maximum percentage germination was reached when a combination of intensity and duration of heat was sufficient to break the dormancy induced by thick seed coats. Moderate temperatures promoted seed germination of *A. gerrardii* while high temperatures caused high seed mortality.

#### 2.4.4 Gases

The gaseous phase of soil occupies those pores that are not already filled with water. Only managing the soil structure and water content can control the amount of air space. Movement of gases through soil is primarily by molecular diffusion. The diffusion pattern may be simple or complex depending on where the seed lies within the soil structure. When a crop seed lies in the intercrumb pores in a newly prepared seed bed it is necessary to consider only diffusion through the macropores. The water held in a cultivated soil at field capacity does little to restrict gas diffusion through the soil in bulk. But, if the seed lies inside the crumb matrix, the diffusion coefficient will be restricted by bonding between particles in the crumb and by water in the crumb pores. At field capacity, when the crumb is saturated, gas diffusion inside the crumb is entirely in solution, giving a drastic increase in diffusion resistance of four orders of magnitude [38]. In waterlogged soils and especially in heavy soils, the oxygen content of the gaseous phase may drop considerably below that in normal air. The gas phase is also influenced by presence of vegetation. Roots of plants will take up oxygen and produce carbon dioxide, changing the balance between the gases. In soils having a high organic content and an active microflora the balance may shift in a similar way. Although oxygen levels in soils rarely drop below 19% and carbon dioxide levels rarely exceed 1%, much greater extremes may occur in microsites such as those adjacent to plant roots or decaying organic matter, and in flooded areas [39,40].

In addition to oxygen, carbon dioxide, and nitrogen, soils may contain several other gases and volatile compounds. Those gaseous compounds are mostly due to anaerobic conditions and the activity of microorganisms. Soils may contain methane,

hydrogen sulfide, hydrogen, nitrous oxide and small amounts of carbon monoxide, ethylene and ammonia [41].

#### 2.4.5 Other factors

Leaf litter, the presence of both deciduous and evergreen trees and interspecific variation in leaf phenology in many tropical forests results in significant spatial heterogeneity on the forest floor due to temporal and spatial variation in litterfall [43, 44, 45].

##### 2.4.5.1 Leaf litter

Spatial variation in the amount of leaf litter covering the forest floor may therefore produce significant heterogeneity, at various spatial scales, in seed survival and seedling establishment [45]. Cintra [46] found that the maximum time of *Astrocaryum* seed survival (number of weeks) was related to levels of litter cover. Therefore, areas in the forest with thicker litter were more likely to be safe for large seeds than areas with shallow litter. This indicated that spatial variation in the presence and amount of litter was important, because it generated spatial heterogeneity, and consequently may have amplified the spectrum of plant regeneration niches [47].

Local variation in leaf litter may also increase the diversity of microsites for plant establishment [47,48] and may thus contribute to the high tree species diversity in tropical forests [45]. Microsite factors, such as light, temperature, moisture and nutrient availability, can be modified by the amount of overlying litter with significant consequences for germination and seedling performance [49]. The mechanical protection of leaf litter cover on seeds could contribute to the creation of

good microsite conditions (humidity and temperature) for seed germination. These two abiotic factors could be critical to the maintenance of seed viability through the sometime-extreme droughts that occur during the dry season [50]. However, variations in the amount of litter present at a given microsite can reduce seedling survival, depending on the plant species and light conditions. For example, significantly greater numbers of *Astrocaryum* seedlings died where there was more leaf litter but this was not true for *Dipteryx* [45].

#### 2.4.5.2 Leaf litter and behaviour of seed predators

The foraging behaviour of mammalian seed predators might be affected by substrate (e.g., presence and amount of litter). Rodents mainly locate their food by olfaction [51], and thick litter could hinder their detection of seeds. The mechanical protection afforded to seeds by leaf litter may be relative and short-lived. Because few plant species fruit during the dry season, vertebrate seed eaters must rely on a few keystone species in tropical forests, such as *Astrocaryum* and *Dipteryx*, to meet their metabolic needs [52]. Food scarcity may prompt seed eaters to invest significantly higher amounts of time in searching during the dry season than in the wet season, and seeds may not survive long periods. In fact, few seeds survived until the end of same experiments [46].

#### 2.4.5.3 Predators: Differences in abundance of mammalian predator-dispersers contributed to the differences in seedling densities among sites and have been shown to have significant impacts on populations of mature annual plants [53].

The survival of fresh seeds, the long-term survival of dormant seeds, and the transition from seed to seedling are important life history processes that may be

influenced by animal dispersers and seed predators as well as by spatiotemporal variation in the abiotic environment [54,55].

A study of the impact of mammalian predators on seedling establishment by Sork [53] found that there was 97% predation of seeds beneath fruiting adults were depredated on Barro Colorado Island. Seed survival at a given site is probably a function of the density of mammalian predator-dispersers, the response of mammalian predators through migration and the changes in home range to fruit and seed availability, and the availability of other food sources. In spite of the high costs of seed predation, mammalian dispersal has some advantages. First, if the seed is buried, it has a higher chance of survival than if it remains above the soil. Second, seeds that are scattered by animals feeding on the fruit have a greater chance of survival than those that remain in high densities near the parent [53].

Plant species with small seeds, as well as those with big seeds that germinate quickly, maybe more likely to escape from predators. Large seeds, such as those of *Astrocaryum* and *Dipteryx*, were more easily found by a mammal scanning at ground level [46].

Inouye *et al.* [56] demonstrated significant effects of predation on survival and reproduction in a community of desert annuals. The increase in area of some species where rodents, ants or both were excluded indicated that both classes of seed predators had a significant impact on the population of mature annual plants. Rodents appeared to prey selectively on large seeds because the larger seeded species all increased in density when rodents were excluded.

Janzen [57] proposed that for many plant species that are vulnerable to seed predation, seedling establishment can occur only if an individual or a population

produces sufficient seeds to satiate the predators so that some seeds escape. The impact of mammalian predator-dispersers also was very strong at the seedling stage. A seedling transplant experiment demonstrated that seedlings with protection for their roots and attached seeds had higher survival than unprotected seedlings. In addition, surviving seedlings that were not killed by the animals often lost their seed, resulting in reduced resources for growth, or were damaged as the animals dug up the seeds [53].

## 2.5 Review of work done in Thailand

Some research in Thailand has focused on the conditions for seed germination. Hardwick et al. [58] who worked in Doi Pui in northern Thailand found that the large *Beilschmiedia* seeds were indeed much less tolerant of exposure to full sunlight and low moisture than the smaller seeds of *Prunus* and *Engelhardia*. Finegan [59] who suggested that pioneer species are more tolerant of drought and exposure to full sunlight conditions than forest species. As pioneer species tend to be smaller seeded [60] it would be expected that a species mean seed size would be a good predictor of its seedling performance in old fields. On an exposed site with short vegetation or bare ground, full sunlight is the most important limiting factor in the dry season [61], so exposure tolerant species such as *Prunus* are most suited. On shadier sites, with dense, tall herbaceous vegetation, dry season drought is the most important limiting factor and thus large seeded, drought tolerant, shade-loving species such as *Beilschmiedia* may survive best.

Kaosa-ard [62] who studied the factors controlling distribution and growth of Teak (*Tectona grandis* Linn. f), found that this species was light demanding or

intolerant of shade. This was the major cause of failure in the natural regeneration (i.e., seed germination and seedling establishment) of this species, the inadequate light at the ground-level in the forest. Kittinanda [63] observed that germination of teak seed in nurseries under natural forest canopies was less than 10%, whereas that in the open nurseries was between 40-50%. Most of seedlings grown under the forest canopies eventually died by the end of the first rainy season. Studies on the growth and development of teak seedlings under shade of different light intensities found that the optimum light intensity, as determined by percentage of full sunlight, was between 75% and 94% sunlight [62].

The effect of rainfall or soil moisture on teak growth, found that teak appears to avoid both very dry and very moist sites. On dry sites where severe drought stress occurs in the hot-dry season, teak is found to be stunted and shrubby probably due to reduced growth and early loss of apical control. On very moist sites, on the other hand, the tree is usually large and fluted and tends to be replaced by a variety of evergreen forest species. A study under controlled environmental conditions, Kaosa-ard [64] found that teak required relatively high soil moisture conditions for its growth and development. The seedlings of this species when grown under the high constant soil moisture for eight weeks were about five times greater, in terms of dry matter production, than those grown under the severe soil moisture stress.

Last, an important influence on seedling establishment or forest regeneration is fire. Fire is an indigenous ecological factor in many tropical forests. Pokaew [65] found that fire mainly killed the old dominant species, thus allowing new species, adapted to grow in these conditions, to colonize the burnt area. The trees of *Lithocarpus elegans*, *Dipterocarpus obtusifolius*, *Quercus kerrii* and *Buchanania*



*latifolia* were more abundant in the non-burnt area. This suggested that seedlings of these important tree species grow better if they were not damaged by fire. Pokaew [65] found that the non-burnt areas had a dense canopy, which had two discernable layers and had no big clear gaps. On the other hand, the canopy in the burnt area, very incomplete canopy layers. This allowed a lot of light at ground level and the growth of grasses, which provided fuel for subsequent fires [65]. Fire did not seem to affect seedling density because seeds that survived at both sites germinated well. However, fire seemed to reduce the number of seedlings able grow up to become adult trees.

## **Chapter 3**

### **Study Area**

#### **3.1 Area description**

##### **3.1.1 Nahaeo National Park and Community forests**

Phuteen Suansai forest (or Nahaeo Natural Park) is located at  $17^{\circ} 28' 40''$  to  $17^{\circ} 34' 35''$  north latitude and  $100^{\circ} 54' 35''$  to  $101^{\circ} 03' 30''$  east longitude . It covers Tambol Laogohok, Tambol Sang Pa and Tambol Nahaeo of Loei province. The total area of Nahaeo National Park is approximately  $113.1 \text{ km}^2$  including the community forests [66]. (Fig 3.1)

##### **3.1.2 Topographic Features**

The topographical features include high mountainous areas mixed with flat valleys. Few areas are suitable for farming. There are not many people living in the area probably due to the low farming potential and distance from markets ( $> 61 \text{ km}$ ). Slopes range from 2% to greater than 50%. There are large forested areas in the west of the park where there are steep slopes with almost no areas flat enough for farming. There are less steep slopes in the east of the park with more flat areas on the hill sides. The entire study area is approximately 600-1400 m above sea level [67].

##### **3.1.3 Climate**

The climate in Amphur Nahaeo is classified as tropical savannah. According to records of temperature and rainfall in the past 9 years (1988-1996), Amphur Nahaeo had the highest average temperatures in April,  $34.9^{\circ}\text{C}$  and lowest

average temperatures were recorded in December, 10.4 °C. The average annual temperature was 22.7 °C. The average annual rainfall was 1075 mm. The seasons are divided into three: [68] (Figure 3.2)

Rainy: May to October

Winter: November to February

Summer: March to May

#### 3.1.4 Rainfall

The rainfall recorded during the period (1988-1996) indicated that September had highest amount of rainfall (288.8 mm), whereas January had the lowest (6.4 mm). September was the month that has highest frequency of rainfall (18 days per month) [68] (Figure 3.2).

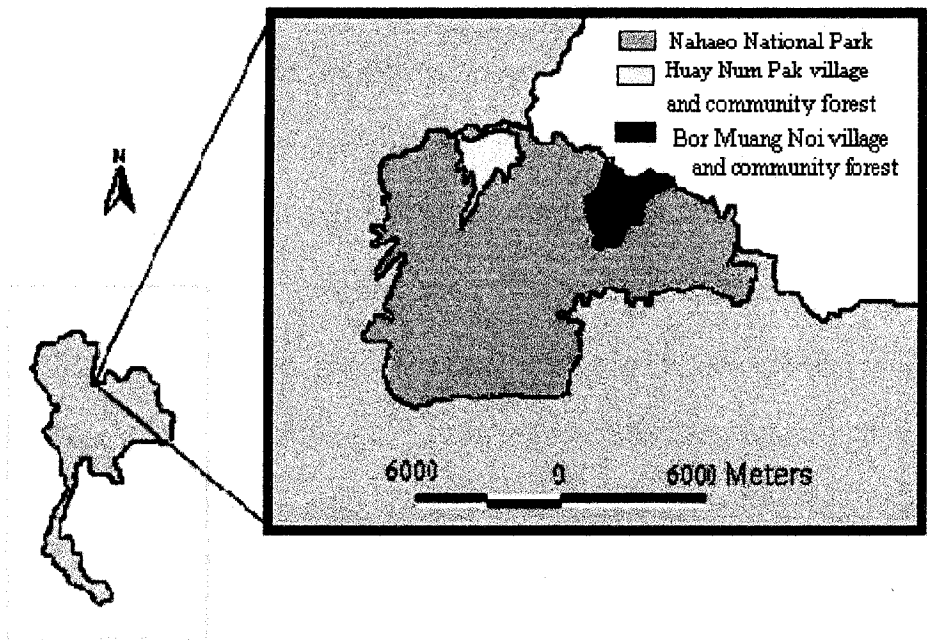


Figure 3.1 : Amphor Nahaeo : The study sites are located within Nahaeo National Park close to Ban Huay Nam Pak and Ban Bor Muang Noi

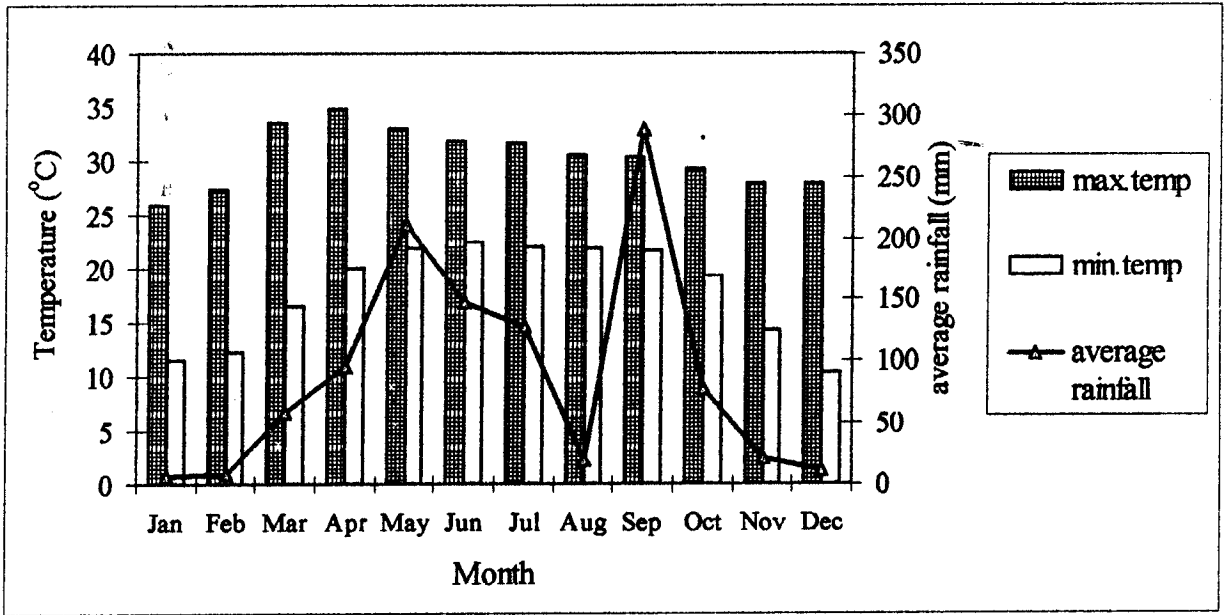


Figure 3.2 maximum temperatures, minimum temperatures and average rain fall between 1988 and 1996 [4]

### 3.1.5 Geological Features

Generally, the area has stoney, steep slopes. Most of it is sandstone. In the areas with hill evergreen forests where the soil is deep, there is no bedrock emerging from the soil. In dry deciduous dipterocarp forest, quartzite is the dominant bedrock. The soils in these areas originated from decayed sands that collapsed and accumulated on plateau areas. Some soils are laterites and soil aggregates mixed with clay. Soils under hill evergreen forest are categorized as fine-loamy, mixed, isohyperthermic oxic palcustults, which are part of the Dan Shai soil group.

### 3.2 Forest types and plant species [3, 66]

Phuteen Suansai forest and forest along the Huang River are located between 500 m and 1,300 m above sea level. The forests are mostly unfragmented and cover an area, and most of it can be described as evergreen forest and semi-evergreen forest with a scattering of bamboo forest. These forests and plant communities within Nahaeo were classified in more detail below.

3.2.1. Evergreen forests. The evergreen forests within Phuteen Suansai forest can be classified into three forest types:

3.2.1.1 Semi-evergreen forest. This forest was found at middle elevations 500-700 m above sea level. The forest has an abundance of canopy trees, mostly evergreen species with a scattering of deciduous trees. The typical major canopy trees included *Irvingia malayanna*, *Hopea odorata*, *H. forea*, *Lagerstroemia calyculata*, *Dipterocarpus turbinatus*, *D. retusus*, and *Alzelia xylocarpa*. The under canopy trees included *Memecylon* sp., *Styrax benzoides*, *Diospyros glandulosa* and *Cinnamomum iners*. The ground vegetation included members of the *Zingiberaceae* and ferns.

3.2.1.2 Hill Evergreen Forest.: Hill evergreen forest was found at 700 m in elevation or above, and it consisted of evergreen trees. The typical canopy trees found in this forest included *Schima wallichii*, *Cinnamomum iners*, *Lithocarpus* sp. and *Quercus* sp. The under canopy trees included *Helicia* sp., *Camellia oleifera* var *confusa*, *Macaranga denticulata*, and *Mallotus paniculatus*. The shrubs included *Melastoma* sp., *Osbeckia chinensis*, *Curcuma* sp., *Alpinia nutans*, *Forrestia* sp., *Commelina* sp., and *Cyanotis* sp. The ferns found included *Pteridium aquilinum*, *Blechnum* sp. and *Brainea insignis* and Epiphytic species.

3.2.1.3. Gallery Forest Gallery forest was found along the Huang River valley and along the small rivers that are found spread all over the National Park. The typical canopy trees found were *Hopea odorata*, *Carallia brachiata*, *Ficus* sp, *Syzygium siamensis*, *Syzygium gratum*, *Bischofia javanica*, *Elacocarpus grandiflorus*, *E. floribundus* and *Duabaga grandiflora*. The under canopy trees included *Globba* sp., *Gnetum* sp. and *Lasia spinos*. Ground cover vegetation included *Equisetum debile*, members of the *Araceae*, and ferns.

### 3.2.2 Deciduous forest

The mixed deciduous forest was found at 700-900 m above sea level and generally was open canopy forest consisting mostly of medium-size trees with a large amount of bamboo in some areas. Most of the trees were leafless in the dry season and forest fires occur almost every year. The typical trees in this forest included *Pterocarpus macrocarpus*, *Alanginum salviifolium*, *Careya sphaerica*, *Croton oblongifolius*, *Terminalia chebula*, and *T. triptera*. The shrubs and ground cover included mostly bamboo and grasses. Epiphytes such as *Hoya* sp. and *Dschidia* sp. were also found on the canopy trees.

## Chapter 4

### Methodology

#### Effects of environmental factors on seed germination and seedling establishment

To investigate the effects of light intensity, vegetation cover, and predators on seed germination and seedling establishment, this study was divided into 2 parts, field and nursery.

##### 4.1 Field study

The effects of canopy cover and ground vegetation cover on *Castanopsis indica* seed germination and seed predation:

##### 4.1.1 Criteria for choosing areas for constructing sample plots.

Areas were chosen in part by the level of *C.indica* use and overall level of human disturbance. First, areas where *C. indica* was used (and which also had higher levels of human disturbance) included the community forests of Ban Huay Nam Pak and Ban Bor Muang Noi. Areas where *C. indica* were not used and generally less disturbed were located in Nahaeo National Park. Within each study area, the sample plots were between 5-10 meters from adults. Locations for constructing sample plots were chosen under the following conditions:

1. Full canopy cover and dense/ ground vegetation cover
2. Full canopy cover and partial or no ground vegetation cover.
3. No canopy cover and dense/ ground vegetation cover
4. No canopy cover and partial or no ground vegetation cover

Sample plots were constructed in each site approximately in proportion to the number of adult trees sampled in each area, (Huay Nam Pak community forest had a low number of sample plots because there was a low number of adult trees used.)

- 18 plots in the National Park
- 8 plots in the Ban Huay Nam Pak community forest
- 21 plots in the Ban Bor Muang Noi community forest

#### 4.1.2 Collecting and selecting *C. indica* seeds

In general, *C. indica* flower in January and fruit in May, and nuts (seeds) fall in September and October. I collected seeds between September and October of 1998 under parent trees.

1. Seeds damaged by insects or other factors were discarded.
2. Seed viability was also tested through floatation [48].

Physical characteristics of *Castanopsis indica* seeds are shown in Table 4.1.

Table 4.1 Physical characteristics of *Castanopsis indica* seeds

Seeds characteristics Average ( $\pm 1$ SD)	
Size	
Width (cm)	$1.6 \pm 0.9$
Length (cm)	$1.8 \pm 1.1$
Weight (g/seed)	$2.6 \pm 0.4$
Color	Brown
Moisture (%) (see appendix I)	$20\% \pm 2.2$

#### 4.1.3 Sample plot construction



### 4.1.3 Sample plot construction

This part of the study compared seed germination between seeds in unprotected sample plots and protected sample plots.

#### Experiment 1 : all seeds unprotected (October 1998- January 1999)

- The locations of sample plots were chosen based on the conditions, outlined in section 4.1.1.
- Plot (1m x 1m) corners were marked using bamboo stakes tied together with rope.
- 25 seeds were selected as in section 4.1.2 for each sample plot. The seeds were distributed evenly throughout the plot.
- The position of each seed was marked by placing a plastic spoon (numbered 1 to 25) next to the seeds at constant intervals depending on the seed density (Figure 4.1).

#### Experiment 2: half the plots were protected with plastic netting (ex 2/1) and the other half unprotected (ex 2/2) (January-March 1999)

- This experiment used the same methods as experiment 1, but only 10 seeds were used per plot (because there were not enough seeds except for plot numbers 11- 18 in the National Park, where 25 seeds were used).
- Huay Nam Pak had 4 plots protected with plastic nets and 4 plots that were unprotected (Figure 4.2).

Bor Muang Noi had 12 plots protected with plastic nets and 9 plots unprotected.

The National Park had 12 plots protected with plastic nets and 18 plots unprotected.

- The seeds were placed at intervals in order to distribute the seeds evenly throughout the plot as above.

Experiment 3: Seeds unprotected (3.1) and protected (3.2) by metal cages (September - November 1999)

- This experiment used the same methods as experiment 1 and experiment 2
- This study only used 10 sample plots in Ban Bor Muang Noi community forest because few seeds were available.
- I used *Castanopsis acuminatissima* (Ko dui) for this experiment because no *C. indica* seeds were available.
- Half of the seeds were protected by metal cages of two sizes (square 5x5x5 cm. and cylindrical 6x 15 cm) to prevent the seeds from being eaten by seed predators (primarily rodents) (Figure 4.3).

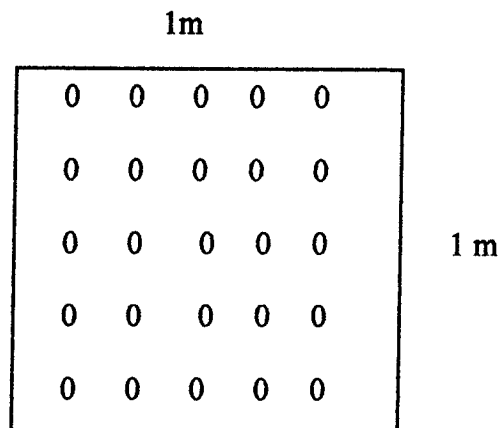


Figure 4.1 Sample plot, 1 square meter in size. The '0' represents seeds and their arrangement in plots with 25 seeds.



Figure 4.2 Sample plots, left, seeds are protected by a plastic net and the plot on the right, seeds are unprotected.

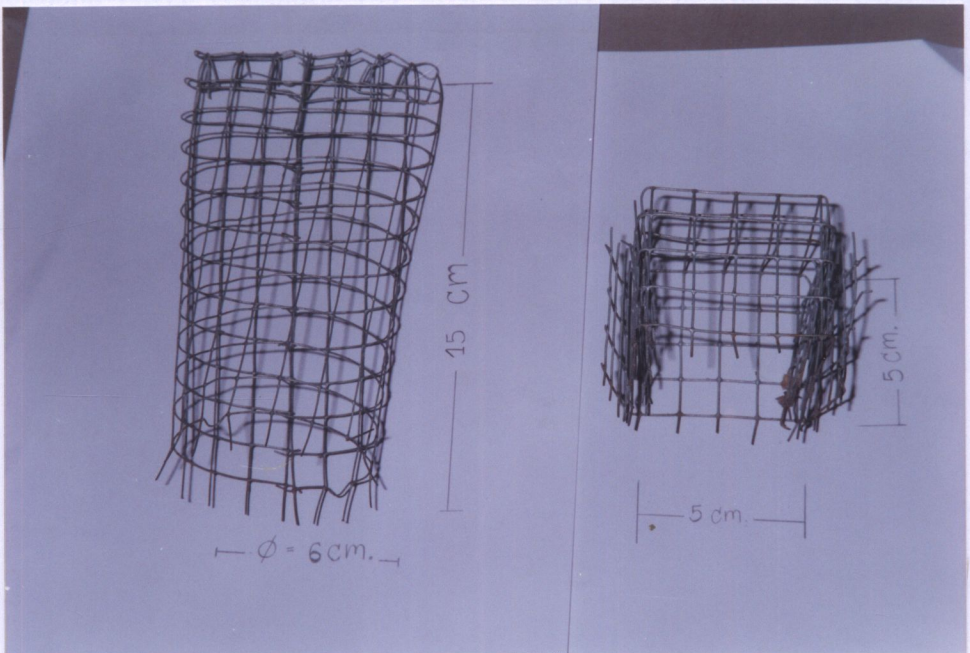


Figure 4.3 Metal cages, 6x15 cm, and 5x5x5 cm.





Figure 4.4 Photograph of seeds covered by metal cages

#### 4.1.4 Methods for measuring ground vegetation cover [69]

The effect of ground vegetation cover on *C. indica* seed germination and seed predation.

Ground cover vegetation (<1 m in height) was measured in each of the sample plots. A sighting tube constructed of PVC plastic, 5.1 cm X 12.7 cm in diameter with 2 strings attached at one end to form cross-hairs (figure 4.5), was used to estimate vegetation cover.

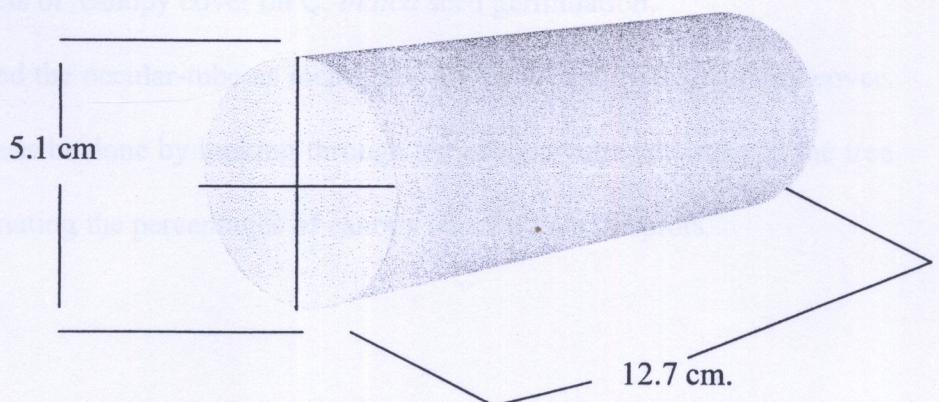


Figure 4.5 Ocular-tube used for estimating the percentage of vegetation cover.



The estimation of vegetation cover was carried out by looking through the ocular-tube held approximately 1 foot from the observer's eye and estimating the percentage of vegetation visible in the circle of the tube. This was done for each quarter of the plot estimated both from the side and from above (Figure 4.6 and 4.7).

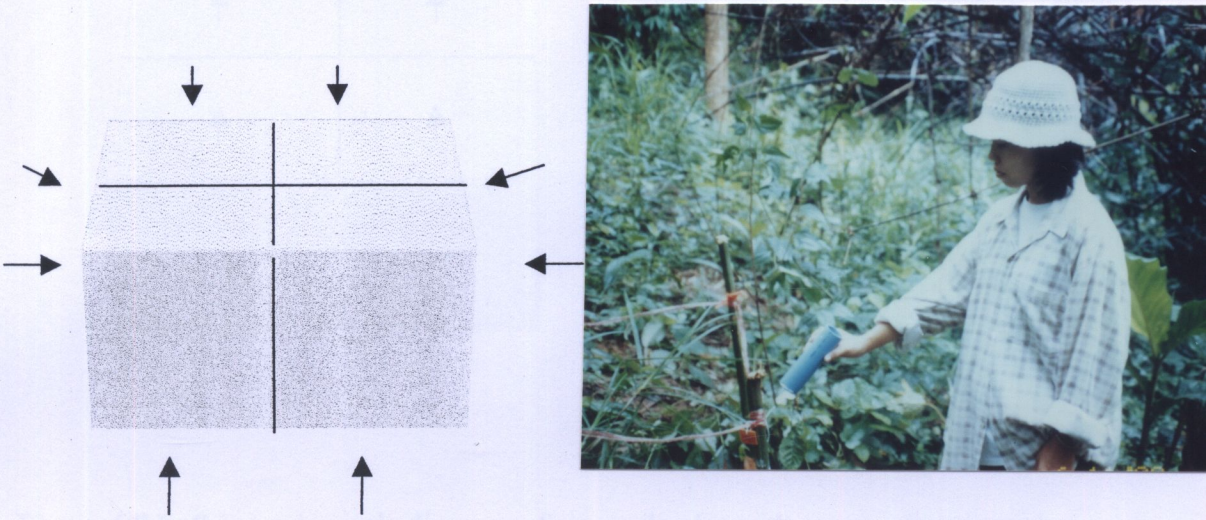


Figure 4.6 Left is a schematic diagram of a sample plot with arrows showing where vegetation was measured. Right, a researcher demonstrates the use of the ocular tube.

#### 4.1.5 Methods for measuring canopy cover [69]

The effects of canopy cover on *C. indica* seed germination.

I also used the ocular-tube as mentioned above for estimating canopy cover. The estimation was be done by looking through the ocular-tube vertically at the tree canopy and estimating the percentages of canopy cover above the plots.



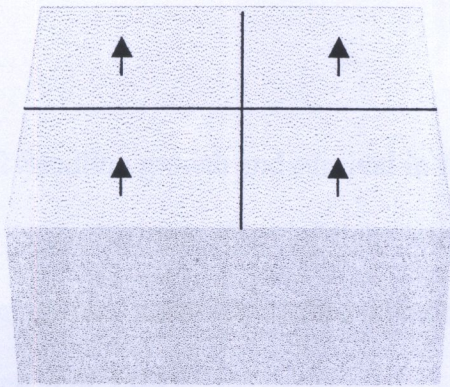


Figure 4.7 Left is a schematic diagram of a sample plot with arrows showing where above which, canopy cover was measured. Right, a researcher demonstrates the use of the ocular tube to measure canopy cover.

4.1.6 The sample plots were checked weekly for 2 months. Each week, the seeds were counted and their status was recorded, such as cause of seed damage and the number germinated.

4.1.7 The results were analyzed by determining the correlation between seed germination (or seed predation), and vegetation cover and canopy cover. The percentage of seed germination (maximum, based on the number of surviving seeds) and the minimum seed germination (percentage of seeds germinated based on the total starting number of seeds) following the methods of Vongcompan *et al.* [70].

Seed germination max. =  $\frac{\text{Number of seeds germinating} \times 100}{\text{Number of remaining seeds}}$

Number of remaining seeds

Seed germination min. =  $\frac{\text{Number of seeds germinating} \times 100}{\text{Total starting number of seeds}}$

Total starting number of seeds

#### 4.2 Seedling growth and survival in the nursery

I studied the effect of light intensity and water availability on *C. indica* seedling growth under controlled conditions generally following the methods of Hardwick *et al.* [58].

##### 4.2.1 Soil and seedling preparation

4.2.1.1. I removed approximately 1-year old seedlings of *C. indica* from the forest that were of similar height and stem width (approximately 12 cm tall with a 1.6 mm stem diameter) on 20 December 1999 (Figure 4.8).

4.2.1.2. Seedlings were planted in individual bags (8x25cm) containing forest soil and rice husk to improve water absorption (2:1, soil: husk).





Figure 4.8 Example of seedlings of *Castanopsis indica* used for the nursery experiment.

#### 4.2.2 Experimental design.

I randomly assigned seedlings to be grown in one of three light treatments (full sunlight, partial shade (30% of full sun), and heavy shade (13% of full sun) and two watering treatments (low moisture and high moisture) using a factorial design with 24 seedlings per treatment. Full sun was provided by growing the plants in an open area, which received a minimum of 4 hours of midday sun. Partial and heavy shade treatments were provided by shading the plants with one or two layers of shade cloth respectively. Seedlings in the low moisture treatment were subjected to natural rainfall only, while high moisture treatments received supplementary water two times per day in the full sun treatment, once per day in the partial shade and in the heavy shade every other day following the methods of Hardwick *et al* [58].



4.2.3 The growth rates were checked weekly for the first two months; the number of surviving seedlings was counted and vertical growth, radial growth and leaf area were measured (see below);

Height : the maximum vertical height of each seedling was measured with a tape measure to the nearest millimeter (from the base of the soil to the shoot apex) (Figure 4.9) .

Radial Growth : Calipers were used to measure (to the nearest 0.1 mm.) stem diameter at ground level or at the top of the root collar [71] (Figure 4.10).

Leaf Area : length and width of healthy leaves were measured to the nearest mm with a tape measure. Area of healthy leaves was estimated using the following equation (see Appendix II for a description of leaf area calculations):

$$\text{Leaf Area} = (A * X) + (B * Y) + (C * Z)$$

Where      A= length (cm)  
               B= width (cm)  
               C= length (L) \* width(W) (cm<sup>2</sup>)  
               X= length coefficient = 0.21  
               Y= width coefficient = 1.08  
               Z= L\*W coefficient = 0.52



Figure 4.9 An example of how seedling height was measured



Figure 4.10 Measurement of stem diameter

4.2.4 I calculated relative growth rate (RGR) (% / year, for vertical, radial and leaf area growth) and a Relative performance Index (RPI, combining survival and growth rate in one index) following the methods of Zagkum *et al.* [72].

$$\text{RGR} = \frac{(\text{LnH}_2 - \text{LnH}_1) \times 365 \times 100}{(t_2 - t_1)}$$

where  $H_1$  = (height, radial diameter or leaf area; cm, mm or  $\text{cm}^2$ ) on the start day

$H_2$  = (height, radial diameter or leaf area; cm, mm or  $\text{cm}^2$ ) on the final day

$(t_2 - t_1)$  = time between first and last measurements (days)

$$\text{RPI} = \frac{\text{mean \% Survival} \times 10}{\text{Max. mean \% Survival}} \times \frac{\text{mean RGR} \times 10}{\text{Max. mean \% RGR}}$$

4.2.5 I assessed the correlation between light intensity, water availability, and leaf area against seedling RGR and RPI. Differences among treatments were assessed using t-tests. Differences in mortality were assessed using  $\chi^2$  (Chi-square) tests.

4.2.6 The seedlings were measured for a total of 260 days (25 January to 12 October 2000).

### 4.3 Seedling growth and survival in the field

4.3.1 Plots were chosen near (<50 m) from where seedlings for the nursery study were collected (seedling also had similar heights and stem widths to those used in the nursery experiment).

4.3.2 The locations were chosen randomly for 5, 2x2 m plots.

4.3.3 Bamboo stakes were used to mark the four corners of the plot as above.

4.3.4 Five seedlings per plot were randomly chosen for measurement which included height, stem radius and leaf area following the same methods as with the seedlings in the nursery.

4.3.5 These data were compared with the seedlings grown in the nursery.

4.3.6 The seedlings were also measured for a total of 260 days (25 January to 12 October 2000).

## CHAPTER 5

### Results and Discussion

As noted in Chapter 4, I divided the experiment into two parts, part one: seed germination and seed survival and part two: survival and growth of seedling in the nursery.

#### Part 1: Seeds

##### 5.1. Effect of canopy cover and vegetation cover on seed germination

###### 5.1.1 Germination

The overall germination was low for all experiments, ranging from 1.7% to 13.0% (Table 5.1). To determine if canopy cover effected germination, canopy cover above the field plots was divided into two equal groups. A plot was considered to have an “open” canopy if the cover was less than 23.8%. The canopy was considered closed if the cover was equal to or greater than 23.8%. Ground vegetation was also divided into two groups. Cover was called “low” if it was less than 28.5%. The vegetation cover was considered “high” if it was equal to or greater than 28.5%.

During the first experimental period (October 1998- January 1999), percent seed germination was highest in plots with a low percentage of ground vegetation cover and high percentage of canopy cover, but these differences were not statistically significant ( $\chi^2$  test,  $P > 0.05$ ). Germination was not significantly different between high and low vegetation cover, but was significantly different in terms of canopy cover ( $\chi^2$  test,  $P < 0.05$ ) (Table 5.1). During the second experiment there were no significant differences in seed germination in relation to canopy cover or

vegetation cover (Table 5.1). The percentage of seeds germinating during experiment 1 was significantly greater than experiment 2/2 (t-test,  $P < 0.05$ ). There were no other significant differences among these three experiments (Table 5.2).

Table 5.1 Effect of canopy cover and ground vegetation cover on seed germination

	Experiment 1				Experiment 2/2			
	number of seeds starting		number of seeds germinating		number of seeds starting		number of seeds germinating	
	open canopy *	closed canopy **	open canopy	closed canopy	open canopy	closed canopy	open canopy	closed canopy
low vegetation cover	375	200	7[1.9%]	26[13.0%]	140	60	4[2.8%]	1[1.7%]
high vegetation cover	200	400	11[5.5%]	16[4.0%]	30	50	0	2[4.0%]

1. Experiment 1 October 1998- January 1999 (plots without nets)

2. Experiment 2/2 January 1999- March 1999 (plots with nets, seeds stored 3 months prior to the experiment)

\* See text for definition of open and closed canopy

\*\*Experiment 1 germination was significantly different between "open" and closed canopy (t-test,  $p < 0.05$ ), but there was no difference between the two groups of ground vegetation cover.

Experiment 2 germination was not significantly different among the different levels of canopy cover and vegetation cover (t-test,  $p > 0.05$ )

Table 5.2 Percentage of seed germination for experiment 1, experiment 2/1 and experiment 2/2. Germination percentages with the same letters were not significantly different, t-test,  $P > 0.05$

	Experiment 1	Experiment 2/1	Experiment 2/2
Mean % germination	4.4 a	2.4 ab	0.8 b

1. Experiment 1 October 1998 – January 1999 (plot without nets)

2. Experiment 2/1 January 1999 – March 1999 (plot with nets, seeds stored 3 months prior to the experiment)

3. Experiment 2/2 January 1999 – March 1999 (plot without nets, seeds stored 3 months prior to the experiment)



### 5.1.2 Seed mortality

Seed mortality was high for all experiments, ranging from 64.0% to 92.5% (Table 5.3). During the first experiment, seed mortality was slightly higher under the more closed canopy and higher vegetation cover, compared to other treatments, but these differences were not statistically significant ( $\chi^2$ -test,  $p > 0.05$ ). Results from both the first and second experiments suggested that most of the mortality was due to mammalian predators (Table 5.4). During the second experiment seed mortality was highest under the more closed canopy with low vegetation cover. These differences were not significant ( $\chi^2$ -test,  $P > 0.05$ ) (Table 5.3).

Table 5.3 Effect of canopy cover and ground vegetation cover on seed mortality

	Experiment 1				Experiment 2/2			
	number of seeds starting		number of seeds removed*		number of seeds starting		number of seeds removed	
	open canopy	closed canopy	open canopy	closed canopy	open canopy	closed canopy	open canopy	closed canopy
low vegetation cover	375	200	342[91.2%]	126[63.0%]	140	60	109[77.0%]	52[86.7%]
high vegetation cover	200	400	184[92.0%]	370[92.5%]	30	50	21[70%]	32[64.0%]

1. Experiment 1 October 1998- January 1999 (plots without nets)

2. Experiment 2/1 January 1999- March 1999 (plots with nets, seeds stored for 3 months prior to the experiment)

\* removed or eaten by predators this included seeds that were eaten after germination

Experiment 1 germination was not significantly different among the different levels of canopy cover and vegetation cover ( $\chi^2$ -test,  $p > 0.05$ )

Experiment 2 germination was not significantly different among the different levels of canopy cover and vegetation cover ( $\chi^2$ -test,  $p > 0.05$ )

Table 5.4 Effect of ground vegetation cover on seed predation

	Experiment 1		Experiment 2/2	
	number of seeds starting	number of seeds removed*	number of seeds starting	number of seeds removed*
low vegetation cover	575	540 [93.9%]	200	122 [61.0%]
high vegetation cover	600	585 [97.5%]	80	23 [28.8%]

1. Experiment 1 October 1998- January 1999 (plots without nets)

2. Experiment 2/1 January 1999- March 1999 (plots with nets, seeds stored for 3 months prior to the experiment)

\* Removed or eaten by predators

Experiment 1 germination was not significantly different among the different levels of canopy cover and vegetation cover ( $\chi^2$ -test,  $p > 0.05$ )

Experiment 2 germination was not significantly different among the different levels of canopy cover and vegetation cover ( $\chi^2$ -test,  $p > 0.05$ )

This study suggested that the effects of vegetation cover and canopy cover on seed germination was small probably because the seed mortality was so high (Table 5.3 and 5.4). Vegetation cover had some effect on mammalian predation during the first experimental period (October 1998- January 1999). Predation appeared to increase with increasing ground vegetation cover (Figure 5.1). Analyzing each forest separately, I found that only in Bor Muang Noi did seed predation increase with increasing vegetation cover although this relationship did not reach statistical significance (Figure 5.2). The other forests (Huay Nam Pak and National Park) showed no significant effects. During the second experimental period there were no significant effects of ground vegetation cover.

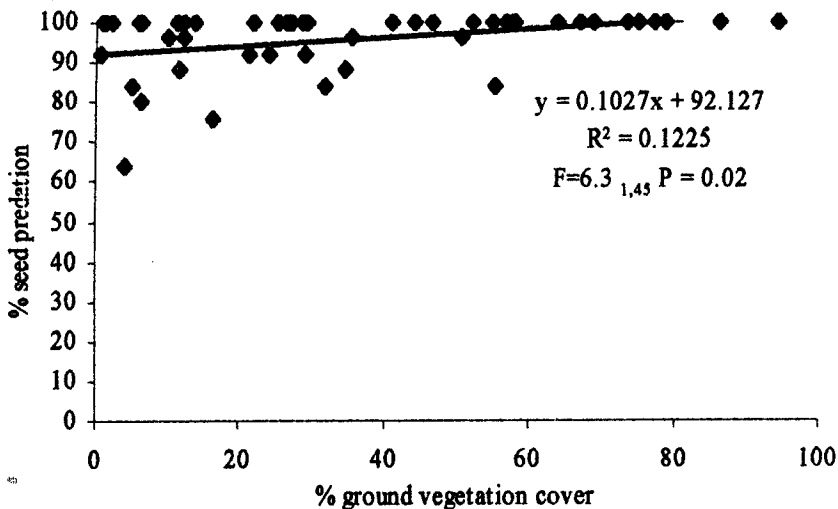


Figure 5.1 Effect of vegetation cover on seed predation in experiment 1 in all forests combined (October 1998- January 1999, plots without nets)



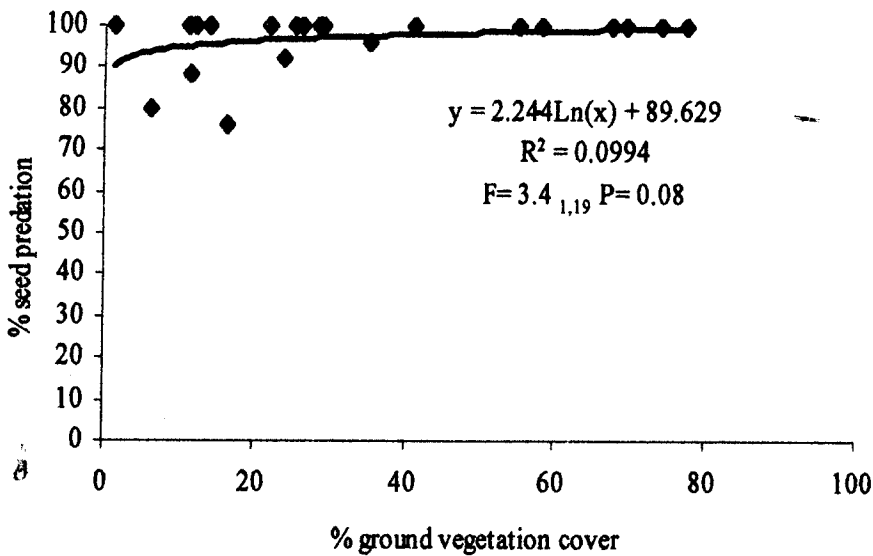


Figure 5.2 Effect of vegetation cover on seed predation during experiment 1, Bor Muang Noi forest (October 1998- January 1999, plots without nets)

Because *Castanopsis indica* seeds are large, they are probably a significant food source for seed eaters such as rodents. For example, Cintra [46] found that 98% of seeds were eaten by seed predators. Inovye [56] also found that rodents appeared to prey selectively on large seeds because the larger seeded species all increased in density when rodents were excluded. In my study (experiment 1 and 2) most of the discarded seed coats were found near the sample plots suggesting that small rodents were eating the seeds and not caching them.

The results from the second experimental period (January 1999 – March 1999) using the protective nets were inconclusive due to low germination rates probably caused by the long seed storage period and high predation rates on some treatments; the nets were not effective in keeping mammalian predators away from the seeds. Germination was not significantly different between high and low vegetation cover, or high and low canopy cover.

## 5.2 Germination of *C. indica* and predation on *C. indica* between the community forests and National Park

### 5.2.1 Germination

The percentage of seed germination during experiment 1 (October 1998- January 1999) was highest in the National Park. The seeds that germinated did so in less than two months. But, in experiment 2/1 and 2/2 seeds had lower germination rates (both with nets and without nets) compared to experiment 1 (Figure 5.3).

### 5.2.2 Mortality

The percentage of seed predation during experiment 1 (October 1998- January 1999) was highest in the community forests. In Huay Nam Pak there was 100% predation within 30 days (Figure 5.4). During experiments 2/1 and 2/2 (January 1999- March 1999, plots with nets and without nets) seed predation was highest in the National Park (Figure 5.5 and 5.6).

Sork [53] found that seed survival at a given site was probably a function of the density of mammalian predator-dispersers and that they change their home range to fruit and seed availability, and the availability of other food sources. In this study, it is possible that the agricultural fields adjacent to the community forests served as an alternative food source. Wattanasirungkul's [4] study of agricultural practices of the villages of Bor Muang Noi and Huay Num Pak found that during April and September people cultivated corn and ginger. During April through June and October to December they cultivated peanuts, and rice was cultivated during July through November. Between January and April fields were left fallow.

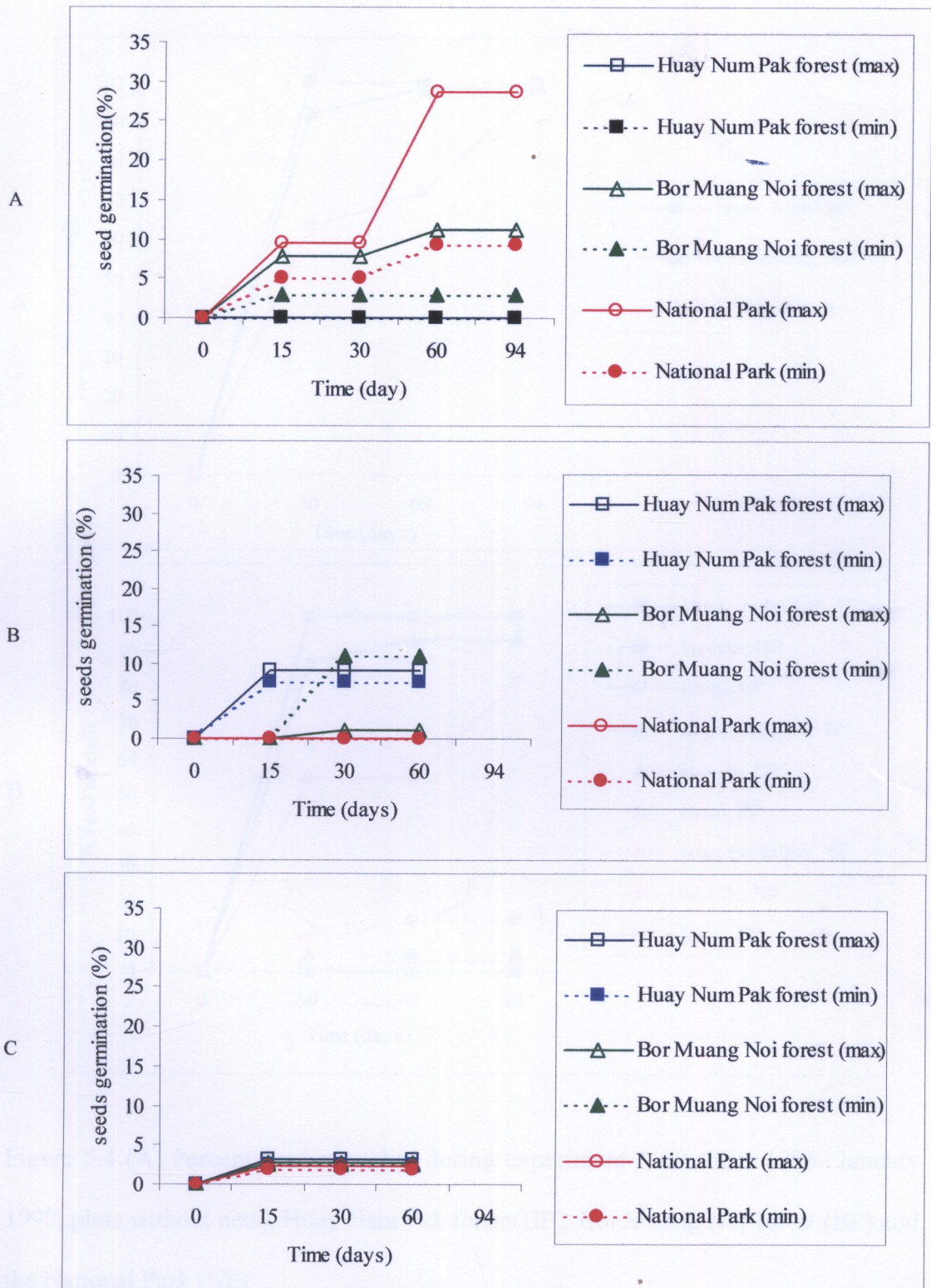


Figure 5.3 Percentage seed germination during experiments 1, 2/1, 2/2

A= Experiment 1, 1 October 1998- 2 January 1999, 94 days (plots without nets)

B= Experiment 2/1, 9 January 1999- 10 March 1999, 60 days (plots with nets)

C= Experiment 2/2, 9 January 1999- 10 March 1999, 60 days (plots without nets)

\* Maximum germination

\*\* Minimum germination



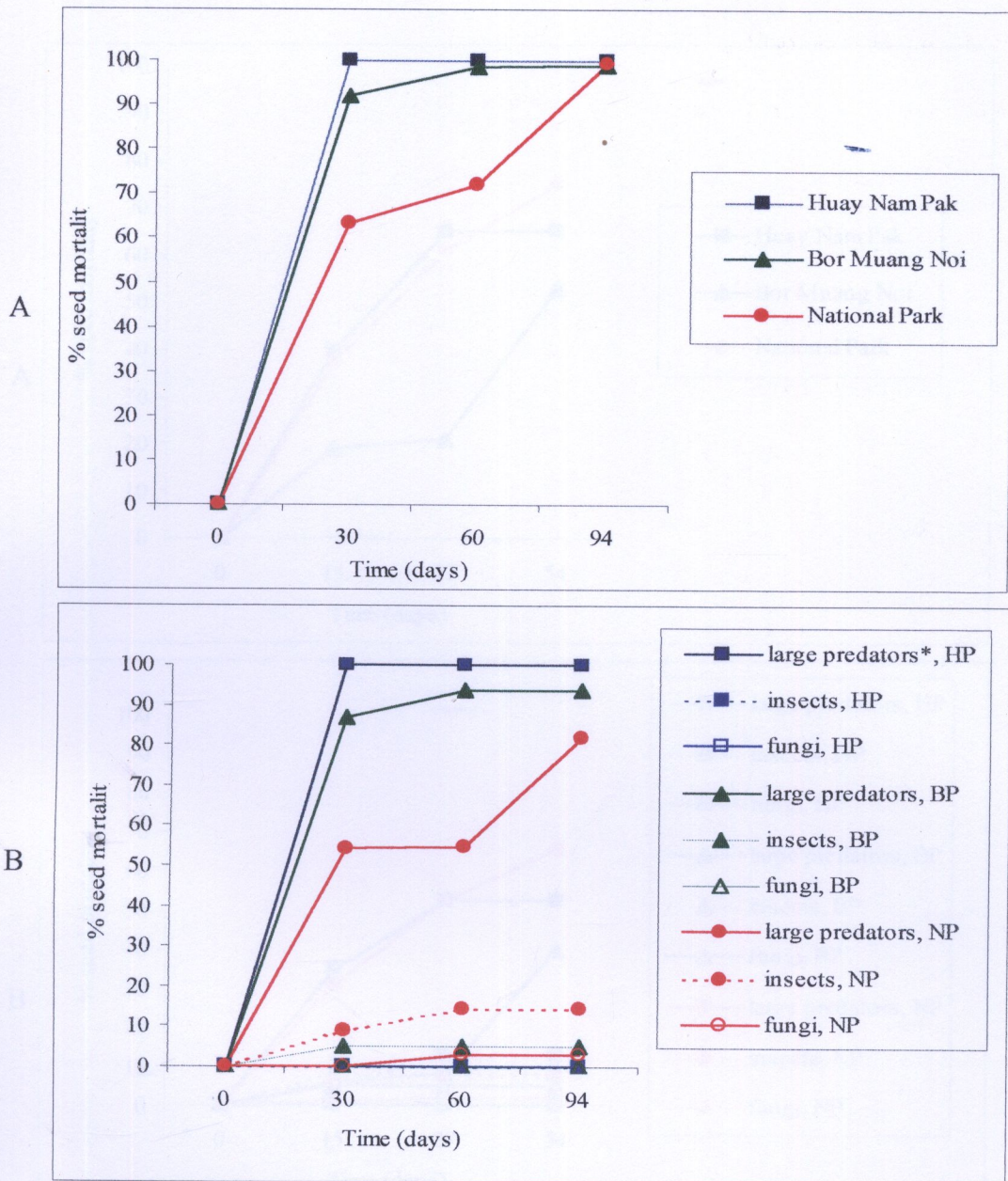


Figure 5.4 (A) Percent seed mortality during experiment 1 (October 1998- January 1999, plots without nets), Huay Nam Pak forest(HP), Bor Muang Noi forest (BP) and the National Park (NP)

(B) Percent seed mortality during experiment 1 by 3 sources, large predators\* (animals other than insects), insects and fungi in the 3 forests.



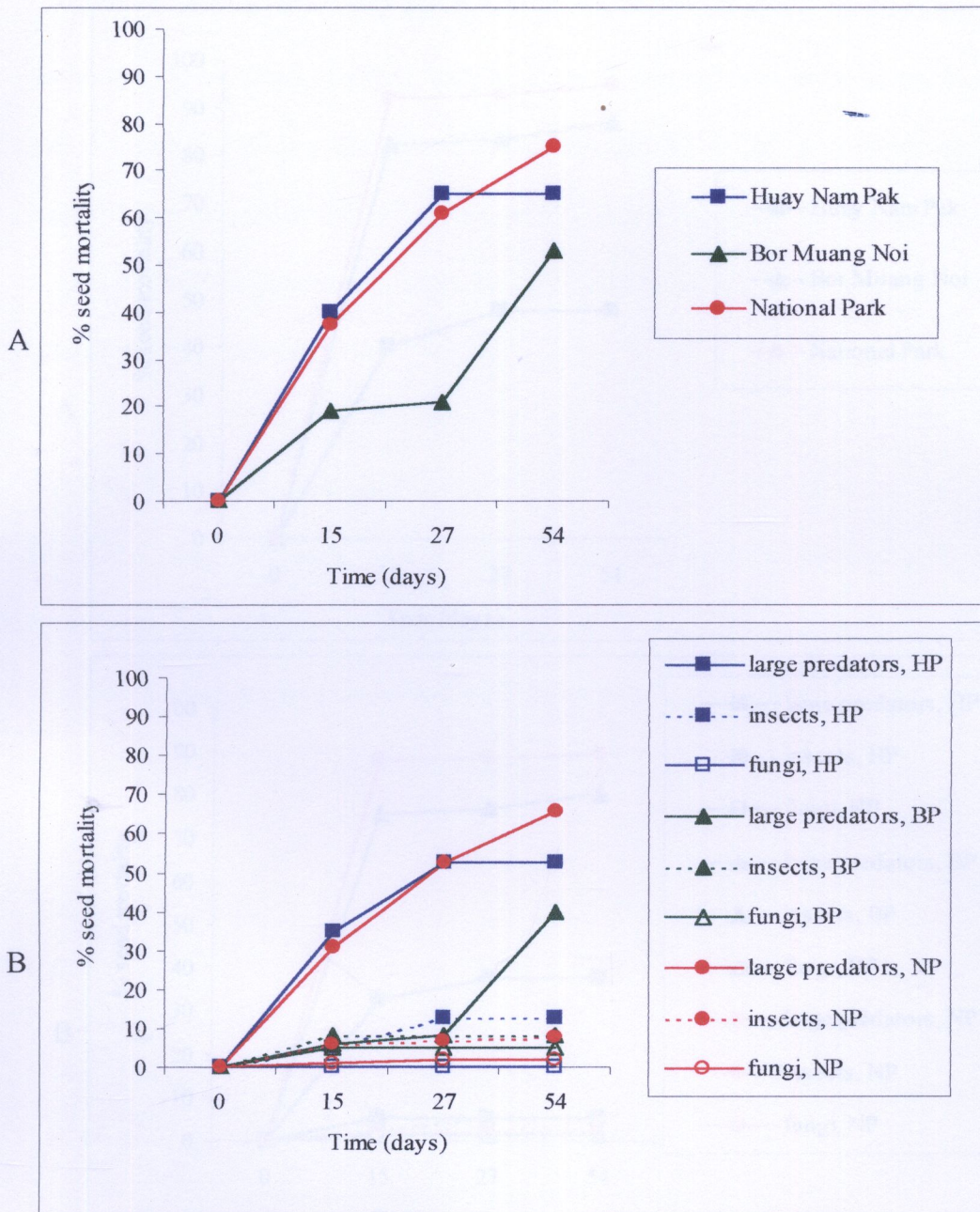


Figure 5.5 (A) Percent seed mortality during experiment 2/1 (January 1999- March 1999, plots with nets), for all 3 forest plots.

(B) Percent seed mortality during experiment 2/1 from three sources, large predators, insects and fungi in the 3 forests.



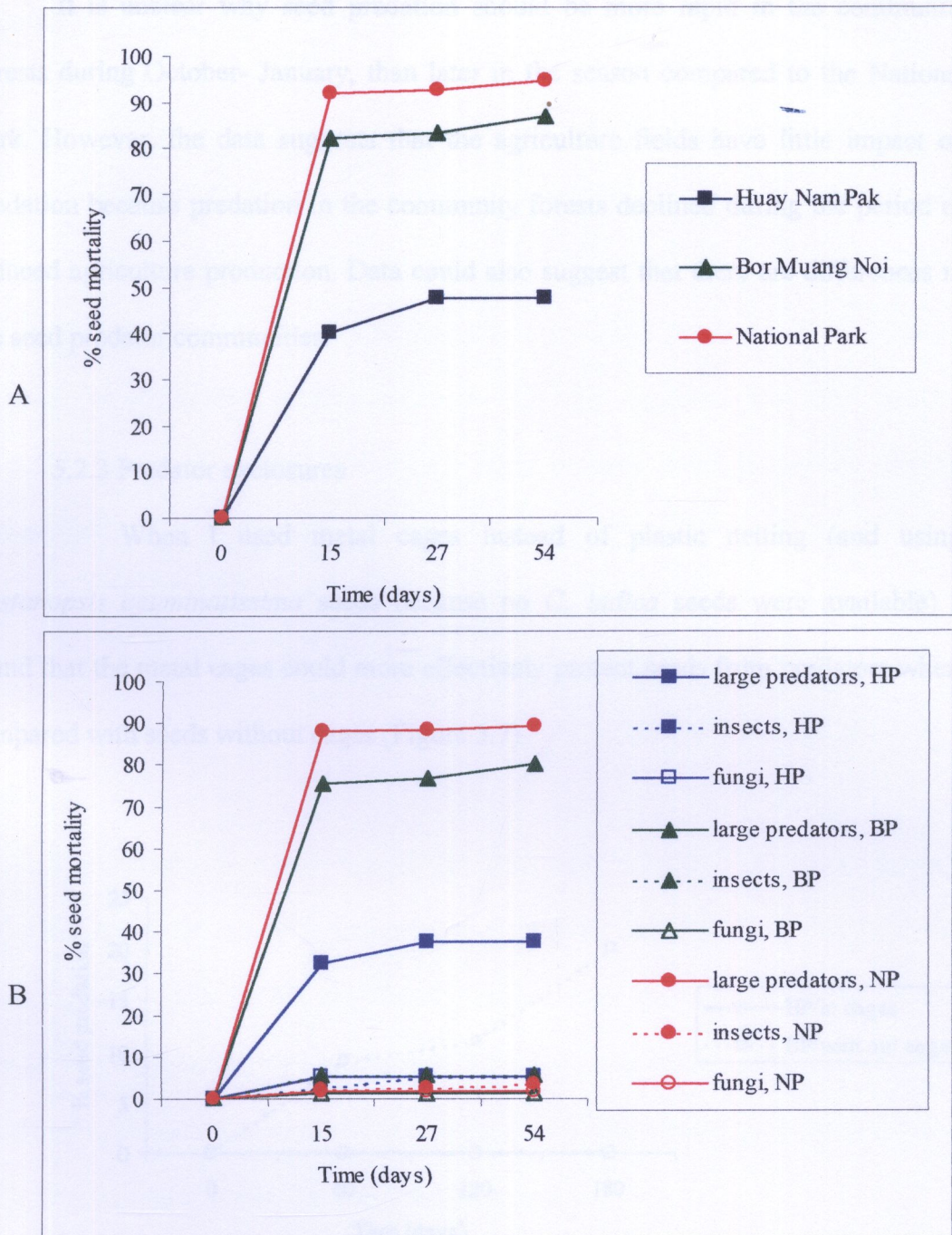


Figure 5.6 (A) Percent seed mortality during experiments 2/2 (January 1999- March 1999, plots without nets), for all 3 forest plots.

(B) Percent seed mortality during experiment 2/2 from three sources large predators, insects and fungi in the 3 forests.

It is unclear why seed predation should be more rapid in the community forests during October- January, than later in the season compared to the National Park. However, the data suggests that the agriculture fields have little impact on predation because predation in the community forests declined during the period of reduced agriculture production. Data could also suggest that there are differences in the seed predator communities.

### 5.2.3 Predator exclosures

When I used metal cages instead of plastic netting (and using *Castanopsis acuminatissima* seeds because no *C. indica* seeds were available) I found that the metal cages could more effectively protect seeds from predators when compared with seeds without cages (Figure 5.7).

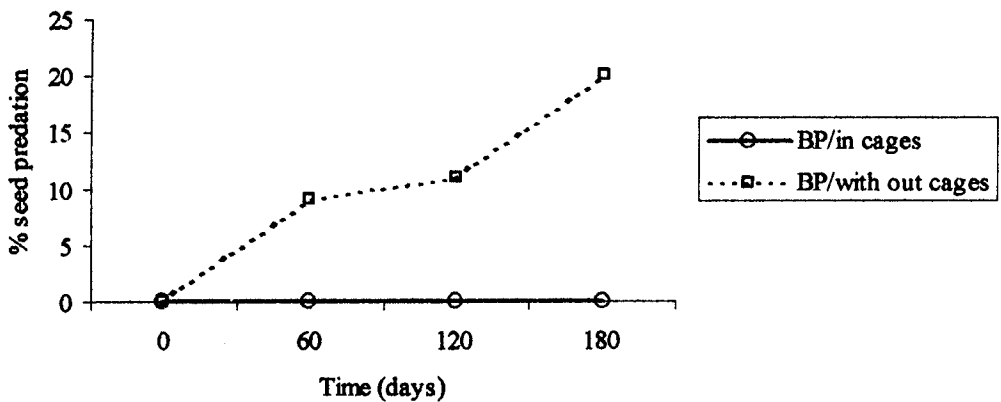


Figure 5.7 Effect of metal cages on seed predation during 180 days at the Bor Muang Noi forest (BP)

## Part II - Seedlings

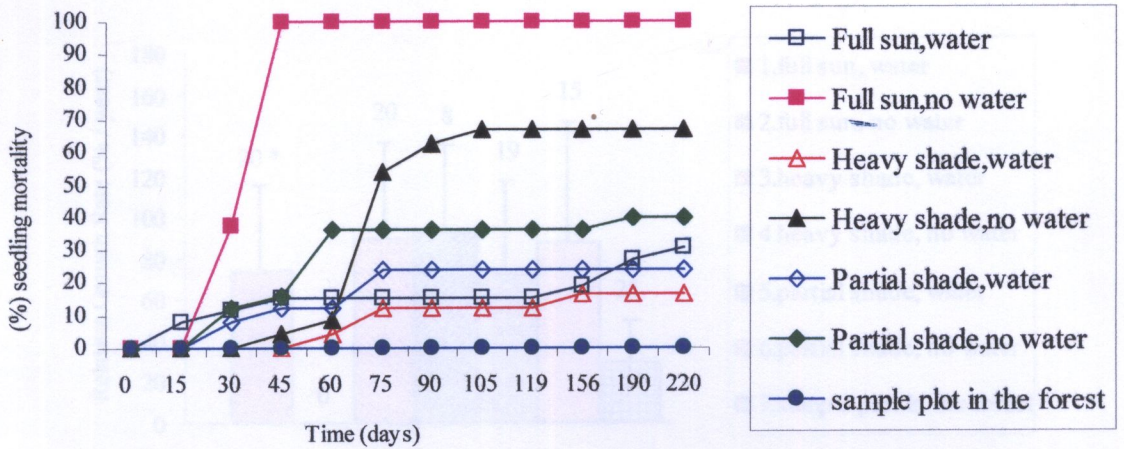
### 5.3. Effects of light intensity and water availability on *C. indica* seedling growth

Treatment 2 (full sun, no water) had the highest mortality rate; all died after 45 days (Figure 5.8). Treatments 4 and 6 (heavy shade, no water and partial shade, no water) had significantly lower mortality rates than treatment 2. This result supports the conclusions of Nepstad *et al.* [73] who suggested that large seeded, shade-tolerant, slow growing species are fairly drought resistant, as they use the energy reserves in their seeds to develop deeper root systems, which tap deep soil moisture reserves. Thus, some shade dramatically increased the survival of these seedlings and therefore areas of open canopy in the forest (such as gaps created by branch and tree cutting) may significantly increase the death rate of *C. indica* seedlings.

The effects of water availability and light intensity on radial growth suggested that all the surviving treatment in the nursery (treatments 1,3,4,5, & 6) had relative growth rates (RGR) significantly higher than the seedlings in the forest (Figure 5.9). Treatment 4 (heavy shade, no water) was significantly slower when compared with treatment 6 (partial shade, no water). The relative performance index (RPI) of treatment 6 was also higher than treatment 4.

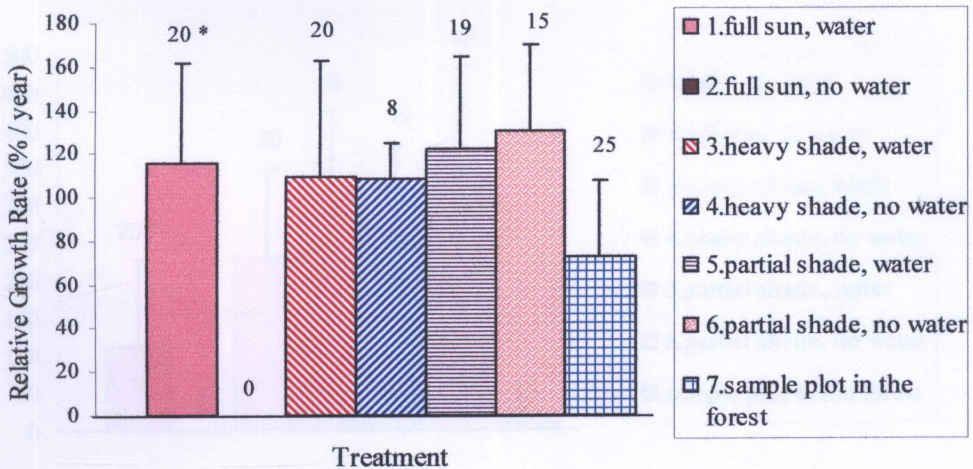
The effects of water availability and light intensity on the relative height growth also suggested that all the surviving seedlings in the nursery treatments (1, 3, 4, 5 and 6) were significantly faster than the sample plot in the forest (Figure 5.10). This result is in partial agreement with other studies, such as Agyeman *et al.* [74] who found that shade tolerant plants may show greater activity in the apical meristem, leading to shorter plants with more slender stems under shade conditions.





Treatment 1=Full sun, watered n=28, dead = 8 (100% light) a (treatments with the same letters were not significantly different,  $\chi^2$ -test  $P > 0.05$ )  
 Treatment 2=Full sun no water n=24, dead = 24 (100% light) e  
 Treatment 3=Heavy shade, watered n=24, dead = 4 (13% light) a  
 Treatment 4=Heavy shade, no water n=24, dead = 16 (13% light) bd  
 Treatment 5=Partial shade, watered n=25, dead = 6 (30% light) a  
 Treatment 6=Partial shade, no water n=25, dead = 10 (30% light) ad  
 Treatment 7=Sample plot in the forest n=25, dead = 0 (5% light) c

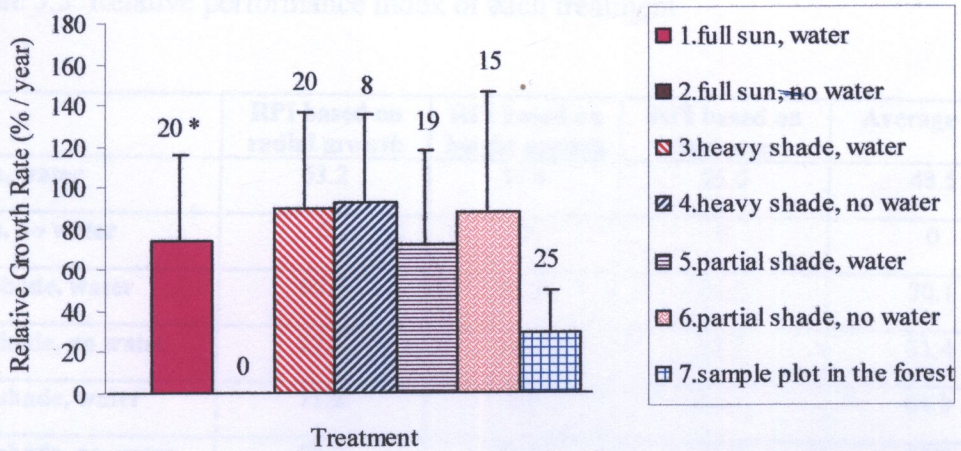
Figure 5.8 Mortality rates of each treatment between January 2000 and 12 October 2000 (220 days)



Full sun, watered, RPI=25.0, n= 28, ab (for treatments with the same letters growth rates were not significantly different, t-test  $P > 0.05$ )  
 Full sun no water, RPI=0, n= 24, e (all dead\*\*)  
 Heavy shade, watered, RPI=60.2, n= 24, ab  
 Heavy shade no water, RPI=33.3, n= 24, a  
 Partial shade, watered, RPI=64.1, n= 25, ab  
 Partial shade no water, RPI=59.9, n= 25, b  
 Sample plot in the forest, RPI=3.3, n= 25, c  
 \* = number of surviving seedlings in each treatment

Figure 5.9 Relative radial growth rate of each treatment between January 2000 and 12 October 2000





Full sun, watered,  
different, t-test  $P > 0.05$ )

Full sun no water,

Heavy shade, watered,

Heavy shade no water,

Partial shade, watered,

Partial shade no water,

Sample plot in the forest,

\* = Number of survival seedlings in each treatment

RPI=57.3, a (for treatments with the same letters growth rates were not significantly

e (all dead \*\*)

RPI=80.2, a

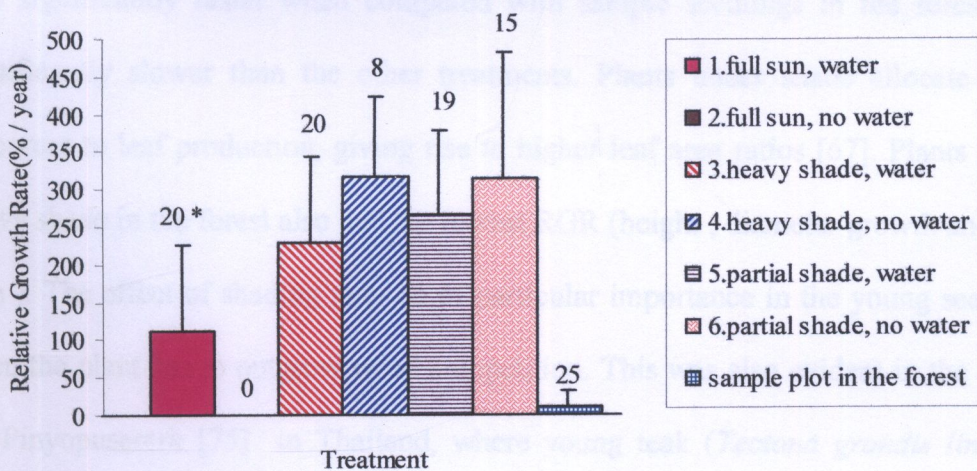
RPI=33.3, a

RPI=58.9, a

RPI=56.7, a

RPI=31.9, b

Figure 5.10 Relative height growth rate of each treatment between January 2000 and 12 October 2000



Full sun and watered, RPI=25.0, a (for treatments with the same letters growth rates were not significantly different, t-test  $P > 0.05$ )

Full sun no water, e (all dead \*\*)

Heavy shade and watered, RPI=60.2, b

Heavy shade no water, RPI=33.3, c

Partial shade and watered, RPI=64.1, bc

Partial shade no water, RPI=59.9, c

Sample plot in forest, RPI= 3.3, d

\* = number of surviving seedlings in each treatment

Figure 5.11 Relative growth rate of leaf area for each treatment between January 2000 and 12 October 2000

Table 5.5 Relative performance index of each treatment

	RPI based on radial growth	RPI based on height growth	RPI based on leaf area	Average RPI
1.full sun, water	63.2	57.3	25.0	48.5
2.full sun, no water	0	0	0	0
3.heavy shade, water	69.9	80.2	60.2	70.1
4.heavy shade, no water	27.6	33.3	33.3	31.4
5.partial shade, water	71.2	59	64.1	64.8
6.partial shade, no water	60.0	56.7	59.9	58.9
7.sample plot in the forest	55.7	31.9	3.3	30.3

The effects of water availability and light intensity on leaf area growth suggested that treatments (3,4,5 and 6) had significantly higher RGR than the sample plot in the forest and treatment 1 (full sun and water) (see Figure 5.15). Treatment 1 was significantly faster when compared with sample seedlings in the forest, but significantly slower than the other treatments. Plants under shade allocate more resources to leaf production, giving rise to higher leaf area ratios [67]. Plants under heavy shade in the forest also had the lowest RGR (height , diameter growth and leaf area ). The effect of shading may be of particular importance in the young seedling when the plant has to outgrow weed competition. This was also evident in the study of Pinyopusarerk [75] in Thailand, where young teak (*Tectona grandis linn. f.*) plants in many plantations grew slowly and, in some cases, died primarily because of heavy shading.

## **Chapter 6**

### **Conclusion**

#### **6.1 Conclusions**

6.1.1 Most (97.5%) of the seed mortality was caused by predation.

6.1.2 The percentage of seed predation increased with increased ground vegetation cover during the early germination period (October-January), but there was no correlation later in the germination period (January-March).

6.1.3 Seed predation also seemed to be slower in the less distributed forest during the early germination period (October- January), and faster later in the season (January-March).

6.1.4 Small metal cages seem to reduce predation better than plastic netting.

6.1.5 Shade (30% of full sunlight) significantly increased seedling survival, under near drought conditions compared with seedling receiving full sunlight.

6.1.6 Decreased shade in forested areas, such as in the community forest may increase seedling mortality during very dry years.

6.1.7 Radial, vertical and leaf growth was significantly less in the forest (5% of full sunlight) compared to the treatments in the nursery. Light availability seems to be the most important factor in regulating growth rates.

6.1.8 The relative performance of seedlings was best in the heavy shade and watered treatment due to moderate growth rates and low mortality.

## 6.2 Limitations of this study and suggestions for future research

6.2.1 Seed germination in experiment 2 was low (Table 5.1), which was assumed to be an effect of low seed viability due to the prolonged (3 months) storage prior to the start of the experiment. Thus, a study of seed storage time should be carried out to examine the effect of seed storage time on seed germination.

6.2.2 The effect of vegetation cover and canopy cover on seed germination was small due to high predation rates (Table 5.3, 5.4). Thus, future studies should build plots that are strong enough to protect the seeds from predators. Different sizes of metal cages also should be used as to examine the effect of cage size on seed predation.

6.2.3 In this study, I did not identify the mammalian seed predators, thus it was difficult to determine why there were differences in predation rates between the community forests and the Natural Park, and why predation rates changed over time.

6.2.4 For the study in the nursery, the number of seedlings per treatment was too small. In addition, their age and conditions under which they grew may have been significantly different before they were transferred to the nursery. Therefore, it would be more useful to use seedlings that had been germinated from selected seeds in the nursery. This would increase the sample size and insure that the seedlings are the same age and have been grown under similar conditions.

## References

1. Bhunibamon, S., 1986, The environmental and socio-economic aspects of tropical deforestation: a case study of Thailand. Department of Silviculture, Faculty of Forestry, Kasetsart University, pp. 102 (in Thai).
2. FAO, 1997, State of the World's Forests 1997, Food and Agricultural Organization of the United Nations, Rome, pp. 66.
3. Nanakorn, W., 1995, A report of the survey of plant species within the area of Huang river and Phu Teen Suan Sai forest, Amphur Nahaeo, Loei province, Botanical Academic Division, Queen Sririkit Botanical garden, Botanical department, pp. 57 (in Thai).
4. Wattanasrirungkul, S., 1999, Study of farming systems for sustainable development; suitable crops for Moo Ban Huay Num Pak, Tumbon Sang Pha, Nahaeo district Loei Province, Master of science thesis, (Natural resource management), King Mongkut's University of Technology Thonburi, pp. 271(in Thai).
5. Potirat, P., 1965, Technology of plantation mushroom, Saharatana Junkasame, pp. 267-275.
6. Gupta, R., Agarwal, M. and Kuman, N., 1996, Effect of lopping intensities on growth and biomass production of *Bauhinia pauhinia* L. in north-west Himalaya low hill region, Indian forester, Vol. 122, pp. 396-404.
7. Eiadthong, V., 1989, A Taxonomic study of the family fagaceae in Haui Kha Kheng Wildlife Sanctuary, Master of science thesis(forestry), Kasetsart University, pp. 1-39 (in Thai).



8. Gardner, S., Sitisuntorn, P. and Anusarnsuntorn, V., 2000, Plant of northern of Thailand, Kothai printing, pp. 545 (in Thai).
9. Kozlowski, T. T., 1972, Seed Biology, Academic Press, New York, pp. 1-21.
10. Bewley, J. D., and Black, M., 1994, Seeds: Physiology of Development and Germination, Plenum press, New York, pp. 273-291.
11. Agyeman, V.K., Swaine, M. D. and Thompson, J., 1999, Responses of tropical forest tree seedlings to irradiance and the derivation of a light response index, *Journal of Ecology*, Vol. 87, pp. 815-827.
12. Vazquez-Yanes, C., and Smith, H., 1982, Phytochrome control of seed germination in the tropical rainforest pioneer trees, *Cecropia obtusifolia* and *Systematics*, *New Phytologist*, Vol.92, pp. 477-485.
13. Taylor, C.J., 1960, Synecology and silviculture in Ghana, Edinburgh, UK., pp. 313-325.
14. Gitay, H., and Noble, I. R., 1997, What are functional types and how should we seek them, *Plant Functional Types*, Cambridge University Press, Cambridge, UK, pp.3-19.
15. Swaine, M.D., and Whitmore, T. C., 1988, On the definition of ecological species groups in tropical rain forests, *Vegetation*, Vol.75, pp.81-86.
16. Kyereh, B., Swaine, M. D., Thompson, J., 1999, Effect of light on the germination of forest trees in Ghana, *Journal of Ecology*, Vol.87, pp. 772-783.
17. Baskin, C.C., and Baskin, J.M., 1998, Seeds Ecology, biogeography, and evolution of dormancy and germination, San Diego, CA, Academic Press, pp.133-136.

18. Hawthorne, W.D., 1993, Forest Regeneration after logging, Chatham Maritime, Natural Resources Institute, pp. 154-160.
19. Hawthorne, W.D., 1995, Ecological profiles of Ghanaian forest trees, UK, Oxford Forestry Institute, pp. 284-299.
20. Holmes, M.G., and McCartney, H.A., 1975, Light and plant development, Butterworths, London, pp. 446-476.
21. Bazzaz, F.A. and Pickett, S. T. A., 1980, Physiological ecology of tropical succession: a comparative review, Annual review of ecology and systematics, Vol. 11, pp. 287-310.
22. Denslow, J. S., 1980, Gap partitioning among tropical rain-forest trees, Biotropica, Vol.12 (Supplement), pp. 47-55.
23. McClure, T. T. and Robbins, W. R., 1942, Resistance of cucumber seedling to damping-off as related to age, season of year, and level of nitrogen nutrition, Botanical Gazette, Vol.103, pp. 684-697.
24. Roth, L. F., and Riker, A. J., 1943, Influence of temperature, moisture, and soil reaction on the damping-off of red pine seedlings by *Pythium* and *Rhizoctonia*, Journal of Agricultural Research, Vol. 67, pp. 273-293.
25. Vaartaja, O., 1952, Forest humus quality and light conditions as factors influencing damping-off, Phytopathology, Vol. 42, pp. 501-506.
26. Goo, M., 1951, Water absorption by tree seeds, Bull Tokyo University Forest, Vol. 39, pp. 55-60.
27. Mayer, A.M., and Poljakoff-Mayber, A., 1963, The germination of seed, New York, McMillan, 236 pp.



28. Stanly, R.G., 1958, Gross respiratory and water uptake patterns in germinating sugar pine seed, *Physiology*, Vol. 11, pp. 503-514.
29. Crocker, W., and Barton, L.V., 1957, *Physiology of seeds*, Waltham, The Chronica Botanica, 210 pp.
30. Goo, M., 1952, When cell division begins in the germinating seeds of *P. thunbergii*, *Journal Japan Forestry Society*, Vol.34, pp.1-3.
31. Edward, D. G. W., 1971, The kinetics of water absorption in stratifying and nonstratifying noble fir (*Abies procera* Pehd) seed, *Canada Journal Forestry Res*, Vol. 1, pp. 235-240.
32. Ching, T.M., 1958, Some experiments on the optimum germination conditions for western hemlock (*Tsuga heterophylla*), *Journal of forestry*, Vol. 56, pp. 277-279.
33. Bonner, F.T., 1968, Water uptake and germination of red oak acorns, *Botanic Gaz.*, Vol.129, pp.83-85.
34. Mayer, A.M., and Poljakoff-Mayber, A., 1966, *Plant Physiology*, The germination of seed, Botany Department, Hebrew, University Jerusalem, Vol.3, pp. 31-59.
35. Hardwick, K., 1992, Factors affecting germination of tree seeds from dry tropical forests in northern Thailand, Chiangmai University, Unpublished.
36. Bhodthipuks, J., Pukittayacamee, P., Saelim, S., Wang, B.S.P., and Yu, S.L., 1995, Rapid viability testing of tropical tree seed, Asean Forest Tree Seed Centre Project, pp. 5-10.

37. Portlock, C.C., Shea, S.R., Major, J.D., and Bell, D.T., 1990, Stimulation of germination of *Acacia pulchella*, a laboratory basis for forest management options, *Journal of Applied Ecology*, Vol. 27, pp. 319-324.
38. Currie, J.A., 1973, *The seed-soil system, seed ecology*, Butterworths, London, pp. 463-480.
39. Roberts, E.H., 1972, *Dormancy: a factor affecting seed survival in the soils, Viability of seeds*, Chapman and Hall, London, pp. 321-359.
40. Egley, G.H., and Duke, S.O., 1985, *Physiology of weed seed dormancy and germination*, Weed Physiology, CRC press, Boca Raton, FL, pp. 27-64.
41. Fenner, M., 1992, *Seeds: the ecology of regeneration in plant communities*, Academic press, New York, pp.339-340.
42. Frankie, G. W., Baker, H. G. and Opler, P. A., 1974, Comparative phenological studies of trees in tropical wet and dry forests in the lowlands of Costa Rica, *Journal of Ecology*, Vol.62, pp. 881-919 .
43. Luizao, F. and Schubart, H. O. R., 1987, Litter production and decomposition in a terra-firme forest of Central Amazonia. *Experientia*, Vol.43, pp. 259-265.
44. Wright, S. J. and Cornejo, F. H., 1990, Seasonal drought and leaf fall in a tropical forest, *Ecology*, Vol.71, pp. 1165-1175.
45. Molofsky, J. and Augspurger, C. K., 1992, The effect of leaf litter on early seedling establishment in a tropical forest, *Ecology*, Vol. 73, pp. 68-77.
46. Cintra, C., 1997, Leaf litter effects on seed and seedling predation of the palm *Astrocaryum murumuru* and the legume tree *Dipteryx micrantha* in Amazonian forest, *Journal of Tropical Ecology*, Vol. 13, pp. 709-725.

47. Grubb, P. J., 1997, The maintenance of species richness in plant communities: the importance of the regeneration niche, *Biological Reviews*, Vol. 52, pp. 107-145.
48. Denslow, J., 1980, Gap partitioning among tropical rain forest trees, *Biotropica*, Vol. 12, pp. 47-55.
49. Facelli, J. M. and Pickett, S. T. A., 1991, Plant litter: its dynamics and effects on plant community structure, *Botanical Review*, Vol. 57, pp. 1-32.
50. Fisher, B. L., Howe, H. F. and Wright, S. J., 1991, Survival and growth of *Virola surinamensis* yearlings: water augmentation in gaps and understory, *Oecologia*, Vol. 86, pp. 292-297.
51. Price, M. V. and Jenkins, S. H., 1986, Rodents as seed consumers and dispersers, Academic Press, New York pp. 191-234.
52. Terborgh, J., 1986, Keystone plant resources in the tropical forest, in *Conservation Biology, The science of scarcity and diversity* (Soule, M, and Wilson, E., eds.), Sunderland, Sinauer, pp. 330-344.
53. Sork, V. L., 1987, Effect of predation and light on seedling establishment in *Gustavia Superba*, *Ecology*, Vol. 68, pp. 1341-1350.
54. Silvertown, J., 1981, Seed size, lifespan and germination date as coadapted features of plant life history, *American Naturalist*, Vol. 118, pp. 860-864.
55. Forvitz, C.C. and Schemske, D.W., 1986, Seed dispersal and environmental heterogeneity in a neotropical herb: a model of population and patch dynamics, The Netherlands, Dordrecht, pp. 169-186.

56. Inouye, R. S., Byers, G. S. and Brown, J. H., 1980, Effects of predation and competition on survivorship, fecundity, and community structure of desert annuals, *Ecology*, Vol. 61, pp. 1344-1351.
57. Janzen, D. H., 1971, Seed predation by animals, *Annual review of ecology and systematics*, Vol. 2, pp. 465-492.
58. Hardwick, K., Healey, J., Elliott, S., Garwood, N. and Anusarnsunthorn, V., 1997, Understanding and assisting natural regeneration processes in degraded seasonal evergreen forest in northern Thailand, *Forest Ecology and Management*, Vol. 99, pp. 203-214.
59. Finegan, B., 1984, Forest succession, *Nature*, Vol. 312, pp. 109-114.
60. Foster, S.A., and Jason, C.H., 1985, The relationship between seed size and establishment conditions in tropical woody plants, *Ecology*, Vol. 66, pp. 773-780.
61. Uhl, C., 1987, Factors controlling succession following slash-and-burn agriculture in Amazonian forest, *Journal of Ecology*, Vol. 75, pp. 377-407.
62. Kaosa-ard, A., 1989, Teak (*Tectona grandis* Linn. f), *Nat. Hist. Bull. Siam soc.*, Vol. 29, pp. 55-74.
63. Kittinanda, S.P., 1963, Natural regeneration of teak in Lampang, *Vanasarn*, Vol. 21, pp. 261-268 (in Thai).
64. Kaosa-ard, A., 1979, Teak Seed Centre, Annual Report No. 1, Teak Seed Centre, Ngao, Lampang, Thailand, pp. 35 (in Thai).
65. Pokaew, C., 1993, The diversity of trees and seedlings in burnt and non-burnt forest, Master of science thesis (Biology), Chiang Mai University, pp. 271 (in Thai).

66. Nahaeo National Park Office, Unpublished, General information of Nahaeo National Park (in Thai).
67. Gale, G. A., Tripetchkul, S. and Pantamas, P., 1999, Unpublished, Investigating Ko Dui (*Castanopsis acuminatissima*) : Case study in Nahaeo National Park, Loei Province.
68. Tongtriphop, P., 1999 , Study of farming systems for sustainable development; suitable crops for Moo Ban Bo Maung Noi , Tumbon Sang Pha, Nahaeo district Loei Province, Master of science thesis, (Natural resource management), King Mongkut's University of Technology Thonburi, pp. 250 (in Thai).
69. James, F. and Shugart, H., 1970, A quantitative method of habitat description, Audubon Field Notes, Vol. 24, pp. 727-736.
70. Vongcompan, S., Elliott, S., Anusarnsuntorn, V. and Maxwell, J. F., 2000, Propagation of Native Forest Tree species for Forestation, Chang Mai University, unpublished.
71. Kobe, R.K., 1999, Light gradient partitioning among tropical tree species through differential seedling mortality and growth, Ecology, Vol. 80, pp. 187-201.
72. Zangkum, S., Elliott, S. and Navakitbumrung, P., 2000, Effects of container type and media on the performance of native tree species to restore degraded forest ecosystems in Northern Thailand. Faculty of Science, Chiang Mai University, Unpublished.
73. Nepstad, D., Uhl, C. and Serrao, E. A., 1990, Surmounting barriers to forest regeneration in abandoned, highly degraded pastures: a case study from Paragominas, Columbia University press, New York, pp. 215-229.

74. Agyeman, V.K., Swaine, M.D. and Thompson, J., 1999, Responses of tropical forest tree seedling to irradiance and the derivation of a light response index, *Journal of Ecology*, Vol. 87, pp. 815-827.
75. Pinyopusarerk, K., 1979, Temperature and light control of growth of Teak (*Tectona grandis linn. F.*) seedlings, Master of science thesis, Australian National University, pp. 200.
76. Kozlowski, T. T., 1972, Essentials of seed testing, Seed biology, Academic press, New York and London, pp. 354-355.
77. Kato, M., Inthavongsa, K. and Imai, K., 1989, An estimation of leaf area in edible canna (*Canna edulis Ker.*), *Japan. Jour. Crop Sci.*, Vol. 58, pp. 753-754.

## Appendix I

### Testing moisture content of seeds [76]

Methods for determining moisture content of seeds may be roughly classified into (a) basic methods in which the moisture is driven out of the seeds by heat and measured by the loss of weight of the original material, (the weight or volume of the condensed moisture), and (b) practical methods designed for rapid routine work and standardized against one or more of the basic methods. Probably all the moisture cannot be driven out of seeds without driving out small amounts of other volatile constituents or causing chemical changes in the material, which would result in weight changes. In applying any method, therefore it is necessary to adhere closely to the prescribed procedure in order that the results of all tests made by that method will be comparable.

The Association of Official Seed Analysis has not published official methods for testing seeds for moisture content. However, the International Seed Testing Association has adopted rules covering most crop species commonly tested. These rules include only two procedures: the air-oven 130 °C method and the air oven 105 °C method. In this study, I used the air-oven 130 °C method.

#### **The 130 °C air oven method**

The air within the oven is at atmospheric pressure and circulated by convection or mechanical means. A temperature of 130 °C and a heating time of 1 hour are specified for most kinds of seeds. The loss of weight that occurs during

drying, calculated on a percentage basis, is taken to be the percentage of moisture in the seed before drying.



## Appendix II

### An Estimation of leaf Area

The measurement of leaf area is necessary to study the production process of crop plants. It is often conducted by the use of several types of automatic area meters. Because of its cost and applicability, alternative methods such as the calculation from measurements of leaf length and leaf width [77]. For *Castanopsis indica*, a non-destructive measurement of leaf area is important for studying its matter production. It is expected that leaf area can be estimated rapidly and accurately as a product of the regression equation taken from leaf length times leaf width because its leaf form is a simple ellipsoid.

### Methods

- 1) I harvested 30 leaves of *Castanopsis indica* of several sizes.
- 2) I used a Calcomp 9100 digitizer with Arc Info software to measure the area of 30 leaves.
- 3) The relationships between the product of leaf length times leaf width and the actual leaf area, and the derived regression equation were examined.

$$\text{Leaf area} = (A * X) + (B * Y) + (C * Z)$$

Where A = length (cm.)

B = width (cm.)

C = length (L) \* width (W) (cm<sup>2</sup>)

X = length coefficient = 0.21

Y = width coefficient = 10.8

Z = L \* W coefficient = 0.52

มหาวิทยาลัยเทคโนโลยีพระจอมเกล้าธนบุรี  
ข้อตกลงว่าด้วยการโอนลิขสิทธิ์ในวิทยานิพนธ์

วันที่ 29 เดือน พฤศจิกายน พ.ศ. 2544

ข้าพเจ้า (นาย/นาง/นางสาว) อัมพรศักดิ์ อธิษฐาน รหัสประจำตัว 4091916  
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หลักสูตร วิทยาศาสตรบัณฑิต สาขาวิชา การศึกษาคณะศึกษาศาสตร์ คณะ ศึกษาศาสตร์ เลขที่ 6  
อยู่บ้านเลขที่ 1-1 / 1 ตรอก/ซอย มหาสารคาม ถนน สาทร แขวง คลองเตย  
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รหัสไปรษณีย์ 10320 ขอโอนลิขสิทธิ์ในวิทยานิพนธ์ให้กับมหาวิทยาลัยเทคโนโลยี  
พระจอมเกล้าธนบุรี โดยมี อ. อัมพร อ. อัมพร ตำแหน่ง คณาบดี คณะศึกษาศาสตร์  
เป็นผู้รับโอนลิขสิทธิ์และมีข้อตกลงดังนี้

1. ข้าพเจ้าได้จัดทำวิทยานิพนธ์เรื่อง The effect of environmental factors on seed germination, and seedling growth and survival of Castanopsis indica  
ซึ่งอยู่ในความควบคุมของ อ. อัมพร อ. อัมพร อ. อัมพร  
ตามมาตรา 14 แห่ง พ.ร.บ. ลิขสิทธิ์ พ.ศ. 2537 และถือว่าเป็นส่วนหนึ่งของการศึกษาตามหลักสูตรของ  
มหาวิทยาลัยเทคโนโลยีพระจอมเกล้าธนบุรี

2. ข้าพเจ้าตกลงโอนลิขสิทธิ์จากผลงานทั้งหมดที่เกิดขึ้นจากการสร้างสรรค์ของข้าพเจ้าใน  
วิทยานิพนธ์ให้กับมหาวิทยาลัยเทคโนโลยีพระจอมเกล้าธนบุรี ตลอดอายุแห่งการคุ้มครองลิขสิทธิ์ตามมาตรา 23  
แห่งพระราชบัญญัติลิขสิทธิ์ พ.ศ. 2537 ตั้งแต่วันที่ได้รับอนุมัติโครงร่างวิทยานิพนธ์จากมหาวิทยาลัย

3. ในกรณีที่ข้าพเจ้าประสงค์จะนำวิทยานิพนธ์ไปใช้ในการเผยแพร่ในสื่อใด ๆ ก็ตาม ข้าพเจ้าจะต้อง  
ระบุว่าวิทยานิพนธ์เป็นผลงานของมหาวิทยาลัยเทคโนโลยีพระจอมเกล้าธนบุรีทุก ๆ ครั้งที่มีการเผยแพร่

4. ในกรณีที่ข้าพเจ้าประสงค์จะนำวิทยานิพนธ์ไปเผยแพร่หรืออนุญาตให้ผู้อื่นทำซ้ำหรือดัดแปลงหรือ  
เผยแพร่ต่อสาธารณชนหรือกระทำการอื่นใด ตามมาตรา 27, มาตรา 28 และมาตรา 29 และมาตรา 30 แห่ง  
พระราชบัญญัติลิขสิทธิ์ พ.ศ. 2537 โดยมีค่าตอบแทนในเชิงธุรกิจ ข้าพเจ้าจะกระทำได้เมื่อได้รับความยินยอม  
เป็นลายลักษณ์อักษรจากมหาวิทยาลัยเทคโนโลยีพระจอมเกล้าธนบุรี

ลงชื่อ อัมพรศักดิ์ อธิษฐาน อัมพรศักดิ์ อธิษฐาน ผู้โอนลิขสิทธิ์  
( อัมพรศักดิ์ อธิษฐาน )

ลงชื่อ อ. อัมพร อ. อัมพร ผู้รับโอนลิขสิทธิ์  
( อ. อัมพร อธิษฐาน )

ลงชื่อ อ. อัมพร อ. อัมพร พยาน  
( อ. อัมพร อธิษฐาน )

ลงชื่อ อ. อัมพร อ. อัมพร พยาน  
( อ. อัมพร อธิษฐาน )