SEED AND SEEDLING PREDATION OF FIVE FRAMEWORK TREE SPECIES IN A DEGRADED FOREST AREA OF BAN NONG HOI, MAE RIM DISTRICT, CHIANG MAI PROVINCE

KHUANPHIROM NARUANGSRI

MASTER OF SCIENCE
IN BIOLOGY

GRADUATE SCHOOL
CHIANG MAI UNIVERSITY
NOVEMBER 2017

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A THESIS SUBMITTED TO CHIANG MAI UNIVERSITY IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN BIOLOGY

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หัวข้อวิทยานิพนธ์ การล่าเมล็ดและต้นกล้าของพรรณไม้โครงสร้าง 5 ชนิด ในพื้นที่ป่าเสื่อมโทรม ของบ้านหนองหอย อำเภอ แม่ริม จังหวัดเชียงใหม่

ผู้เขียน นางสาว ขวัญภิรมณ์ ณะเรืองศรี

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บทคัดย่อ

การฟื้นฟูป่าโดยวิธีการหยอดเมล็ด มีข้อจำกัดหนึ่ง คือ การล่าเมล็ดและต้นกล้าโดยศัตรูตามธรรมชาติ ที่มีทั้งสัตว์มีกระดูกสันหลังและสัตว์ไม่มีกระดูกสันหลัง การศึกษานี้มีวัตถุประสงค์เพื่อหาปริมาณการล่าเมล็ดและต้นกล้าโดยสัตว์มีกระดูกสันหลัง และสัตว์ไม่มีกระดูกสันหลังในพื้นที่ป่าเสื่อมโทรม โดยใช้พรรณไม้ท้องถิ่น 5 ชนิด ได้แก่ หมอนหิน (*Hovenia dulcis*) นางพญาเสือโคร่ง (*Prunus cerasoides*) ฝาละมี (*Alangium kurzii*) มะกอกห้ารู (*Choerospondias axillaris*) และเลือดม้า (*Horsfieldia glabra*) เมล็ดของพืชแต่ละชนิดถูกนำมาหยอดภายใต้ชุดการทดลองที่มีการป้องกันศัตรูตามธรรมชาติ ประกอบด้วย 1) กรงลวด (ป้องกันสัตว์มีกระดูกสันหลัง) 2) การฉีดพ่นยาฆ่าแมลง (ป้องกันสัตว์ไม่มีกระดูกสันหลัง) 3) กรงลวดและการฉีดพ่นยาฆ่าแมลง (ป้องกันทั้งสัตว์มีและไม่มีกระดูกสันหลัง) 4) กรงเปิด และ 5) ชุดควบคุม (สัตว์ทุกชนิดสามารถเข้าถึงเมล็ดได้) มีการเปรียบเทียบเปอร์เซ็นการหายไปของเมล็ด การงอกของเมล็ดและการตายของต้นกล้าระหว่างชุดการทดลอง พบว่าการหายไปของเมล็ดมีความแตกต่างอย่างมีนัยสำคัญระหว่างชนิด เปอร์เซ็นการหายไปของเมล็ดสูงที่สุด สำหรับ เลือดม้าซึ่งเป็นชนิดที่มีเมล็ดขนาดใหญ่ที่สุดและเมล็ดของพืชชนิดนี้ไม่มีการงอกเลย การหายไปของเมล็ด ของพืชอีก 4 ชนิด คือ หมอนหิน นางพญาเสือโคร่ง ฝาละมี และมะกอกห้ารู มีการหายไปของเมล็ดที่ต่ำและไม่แตกต่างอย่างมีนัยสำคัญระหว่างชนิดของพืช ในขณะที่กรงลวดช่วยลดปริมาณการหายไปของเมล็ดได้ ชี้ให้เห็นว่า สัตว์มีกระดูกสันหลังเป็นผู้ล่าเมล็ดที่สำคัญในพื้นที่ศึกษานี้ อย่างไรก็ตามการป้องกันศัตรูตามธรรมชาติไม่ได้ช่วยเพิ่มการงอกของเมล็ด ความแตกต่างระหว่างการงอกของเมล็ดพืชแต่ละชนิดเป็นผลมาจากลักษณะของเมล็ดและปัจจัยที่พืชต้องการในการงอก นอกจากนี้การป้องกันศัตรูตามธรรมชาติสามารถป้องการการตายของต้นอ่อนที่ยังไม่มีใบแท้ (cotyledonous-seedling) แต่ไม่สามารถป้องกันการตายของต้นกล้าที่มีใบแท้ (leafy-

seedling) ได้ ซึ่งชี้ให้เห็นว่าปัจจัยหลักที่มีผลต่อการตายของต้นกล้า คือ ลักษณะของเมล็ด/ต้นกล้า และการแข่งขันกับหญ้าและวัชพืช นอกจากนี้ได้มีการติดตั้งกล้องดักถ่ายสัตว์ เพื่อสำรวจสัตว์เลี้ยงลูกด้วยนมขนาดเล็กและนกที่มีแนวโน้มว่าจะเป็นผู้ล่าเมล็ด/ต้นกล้า มีการใช้กับดักหลุม กับดักกาว และการเก็บตัวอย่างโดยตรง เพื่อเก็บตัวอย่างสัตว์ไม่มีกระดูกสันหลัง สำหรับสัตว์มีกระดูกสันหลัง หนู (*Rattus sp.*) มีความถี่ของการเข้ามาในพื้นที่มากสุด โดยเฉพาะในช่วงเดือนแรกของการหยอดเมล็ด สำหรับสัตว์ไม่มีกระดูกสันหลัง มด (อันดับ Hymenoptera) มีจำนวนมากกว่าสัตว์ไม่มีกระดูกสันหลังกลุ่มอื่น ทั้งหนูและมดมีการรายงานว่าเป็นผู้ล่าเมล็ดในพื้นที่เสื่อมโทรม จากการศึกษานี้ ชนิดที่เหมาะสำหรับใช้ในการหยอดเมล็ด เรียงลำดับตามความเหมาะสมจากมากไปน้อย คือ นางพญาเสือโคร่ง ฝาละมี และมะกอกห้ารู สำหรับ หมอนหิน ได้รับการพิจารณาว่าเป็นชนิดที่มีความเหมาะสมสำหรับการหยอดเมล็ดระดับต่ำ นอกจากนี้ เลือดม้า ไม่แนะนำให้นำมาใช้สำหรับการฟื้นฟูป่าโดยการหยอดเมล็ดโดยปราศจากการป้องกันการล่าเมล็ด

Thesis Title Seed and Seedling Predation of Five Framework Tree Species in a Degraded Forest Area of Ban Nong Hoi, Mae Rim District, Chiang Mai Province

Author Miss Khuanphirom Naruangsri

Degree Master of Science (Biology)

Advisor Dr. Pimonrat Tiansawat

ABSTRACT

One limitation of forest restoration by direct seeding is predation of seeds and seedlings by natural enemies. Natural enemies include both vertebrates and invertebrates. This study quantified seed and seedling predation by both vertebrates and invertebrates in a degraded forest area. Five native tree species were selected; *Hovenia dulcis*, *Prunus cerasoides*, *Alangium kurzii*, *Choerospondias axillaris* and *Horsfieldia glabra*. Seeds of each species were placed on the ground under five treatments; 1) wire cage (vertebrate exclusion), 2) insecticide spraying (invertebrate exclusion) 3) wire cage plus insecticide spraying, 4) open cage and 5) control (no exclusion). Percentage seed removal, seed germination, and seedling (cotyledonous-seedling and leafy-seedling) mortality were compared among the treatments. Seed removal differed significantly among species. Percent seed removal was highest for the *H. glabra*, and seeds of this species germination. Seed removal of four species *H. dulcis*, *P. cerasoides*, *A. kurzii* and *C. axillaris*, was low and percent seed removal did not differ among these species. Caging seeds significantly reduced seed removal, suggesting that vertebrates are major seed predators in this study site. However, excluding predators did not increase seed germination. Differences in germination among species may have been influenced by seed characteristics and germination requirements. Moreover, excluding predators prevented only cotyledonous-seedling mortality but not leafy-seedling mortality. This suggested that other factors such as seed/seedling characteristics and competition with grass and herbaceous weed might be a major cause of seedling mortality. In addition, camera traps were used to identify

which small mammals and birds were present as potential seed/seedling predators. Pitfall traps, sticky traps and direct capture were used to collect invertebrates. Of the vertebrates, rats (*Rattus sp*.) frequently visited the studied site, especially during the first month after seed sowing. Of the invertebrates, ant species (Order Hymenoptera) were more abundant than other invertebrate groups. Both rats and ants have been reported as seed predators in degraded areas. From this study, species recommended for direct seeding, ranked in order of declining performance, were *P. cerasoides*, *A. kurzii*, and *C. axillaris*. *H. dulcis* was considered to be marginally species and *H. glabra* is not recommended without treatments to reduce seed predation.

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LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
χ^2	Chi-Square
CA	Wire cage experiment
cm	Centimeter
CO	Control without protection
CW	Crown width
df	Degree of freedom
e.g.	Example
FORRU-CMU	Chiang Mai University's Forest Restoration Research Unit
g	Gramm
GLM	Generalized linear model
H	Height
IC	Wire cage plus insecticide experiment
IN	Insecticide experiment
m	Meter
ml	Milliliter
MLD	Median length of dormancy time
mm	Millimeter
OC	Open cage experiment

ºC	Degree Celsius
R	Number of photographs per total effort 100 trap days
RCD	Root collar diameter
RGR	Relative Growth Rate
SE	Standard error
SI	Species performance index
T	Treatment

CHAPTER 1

Introduction

1.1 Historical Background

Primary forest areas around the world have been decreasing. Degradation and destruction of forest has accelerated, mostly caused by human activities, particularly agriculture (Chakravarty, 2012). The highest forest destruction rate arose in temperate forests in Asia, Europe and North America in the early 20^{th} century (FAO, 2012). Deforestation and degradation have been linked to changes in climate patterns and biodiversity loss. To maintain biodiversity and mitigate global climate change, forest restoration of degraded areas has been widely recognized as a solution (Parrotta, 2000).

Conventional methods of forest restoration include production of tree seedlings in nurseries and tree planting (Lamb and Gilmour, 2003; FORRU, 2006). Such conventional methods require construction of tree nurseries and intensive care of small seedlings (FORRU, 2006). Seedlings are transported and planted in target areas (FORRU, 2006; Verdone, 2015). This processes is arduous, time-consuming and expensive (FORRU, 2006). Devising forest restoration methods that are cheap and easy to implement will encourage implementation of more restoration projects, leading to increased forest cover.

Direct seeding is an alternative method for accelerating forest recovery by sowing tree seeds directly into deforested areas (Doust *et al*., 2008). It can result in higher seedling performance in the field, compared with conventional tree-planting (Doust *et al*., 2008; Tunjai, 2005; Tunjai and Elliott, 2012), due to better root system development (Doust *et al*., 2008). Direct seeding has been successful in South Africa, but it has not been widely used in tropical Asia (Lamb, 2005).

Major limitations of direct seeding are seed predation and herbivory of seedlings (Holl, 1998; Doust *et al*., 2008). Studies of direct seeding have largely focused on species selection, based on seed characteristics, for example, thickness of seed coat and seed size (Tunjai and Elliott, 2012). I propose that species selection should take into account likelihood of seed removal by predators etc. Therefore the effects of animals on seed removal, germination success and seedling survival should be tested since currently, information on seed removal and seedling predation for direct seeding programs to guide species selection is limited.

Predators of seeds and seedlings, such as insects and mammals usually interact with plants at different life history stages and in different habitats (Fricke *et al*., 2014). At the seed stage, rodents are the most common predators (Hardwick, 1999, Fricke *et al*., 2014; Wood and Elliott, 2003). Large seeds are lost to rodents, but smaller ones are not because they are less easy to find on the ground (Hardwick, 1999). In some degraded areas, ants are major seed predators (Wood and Elliott, 2003). The effects of seed predation and dispersal on plant population dynamics by seed-harvesting ants are strong (Arnan *et al*., 2012). Finally, at the post-dispersal stage, both vertebrates and invertebrates can be major seed predators.

When seeds become seedlings, they are usually attacked by invertebrates, especially insect pests (Doust *et al*., 2008; Fricke *et al*., 2014). Seedlings have low concentrations of defensive chemicals because of their limited photosynthetic area and root biomass, (Orians *et al*., 2010). In tropical forests, most damage by herbivores occurs on young leaves (Kursar and Coley, 2003). Young leaves are more attractive to herbivores, because they lack structural carbohydrates, which contribute to leaf toughness (Wahungu *et al*., 2002). These insects either kill the seedlings outright or seriously reduce their growth and competitive ability (Barton and Hanley, 2013). Most studies focused on seedlings after the development of true leaves. Therefore, we still lack information of predation of early-stage seedlings that have no true leaves.

This study covered three stages of the seed-to-seedling transition, i) seed, ii) cotyledonous seedling and iii) seedling with true leaves.

Four main research questions were addressed.

1) To what extent do invertebrates (especially insects) and vertebrates (small mammals and birds) remove seeds and reduce germination in a degraded site?
2) How much do animals affect seed removal and seed germination?
3) How much do invertebrates and vertebrates affect seedling survival?
4) What are the potential predators at each stage of the seed-to-seedling transition?

1.2 Hypotheses

1) Seed removal and seedling predation

If vertebrates remove seeds and reduce germination, then excluding them will significantly reduce seed removal and increase germination. If invertebrates remove seeds and reduce germination, then excluding them will significantly reduce seed removal and increase germination. Excluding both invertebrates and vertebrates will result in the lowest seed removal.

2) Effects of animals on seed removal and predation

If vertebrates and invertebrates are equally important seed predators, then percent removal and germination of seeds exposed to vertebrates will not be significantly different compared with seed exposed to invertebrates.

3) Seedling predation and survival

If vertebrates are important seedling predators, then excluding vertebrates from seedlings will significantly reduce seedling mortality. If invertebrates are important seedling predators, then excluding invertebrates from seedlings will significantly reduce seedling mortality.

1.3 Research Objectives

1) To determine the intensity of seed and seedling predation by vertebrates and invertebrates in a degraded site.

2) To quantify the effects of seed predation on seed removal and germination.

3) To quantify the effects of seedling predation on seedling survival and growth.

4) To examine the diversity of potential seed and seedling predators.

1.4 Usefulness of the research

1) This study provides a better understanding of natural enemies that are barriers to forest restoration by the direct seeding.

2) The results can be used to improve tree species selection for direct seeding and protective measures against seed and seedling predation can be devised.

3) This study provides knowledges to help with site preparation and management of direct seeding, both before and after direct sowing.

CHAPTER 2

Literature Review

2.1 Forest issues

Forests provide many benefits for humans and wildlife. They supply not only products, but also ecosystem services, such as maintenance of biodiversity, climate and water regulation, and they play a major role in carbon storage (Davies *et al.*, 2013; Percy *et al.*, 2003). More than 4 billion hectares of world's terrestrial area is covered by forests, which constitutes an enormous carbon sink, via photosynthesis and soil storage (Percy *et al.*, 2003; Sedjo, 2001) Because of human population growth and economic development, human activities, such as burning fossil fuels and deforestation emit enormous quantities of CO_2 into the atmosphere, which contributes significantly to rising global temperatures (Sedjo, 2001). Furthermore, demand for land and natural resources has increased, leading to deforestation and depletion of forest resources (Table 2.1) especially in Africa and South America (Chakravarty *et al.*, 2012).

Table 2.1 Global forest cover 1990 to 2010

Regions	**Total forest cover**		
	1990	**2000**	**2010**
	Million Hectares		
Africa	749	709	674
Asia	576	570	593
Europe	989	998	1,005
North and Central America	708	705	705
Oceania	199	198	191
South America	946	904	864
World	**4,168**	**4,085**	**4,033**

Source: Compiled by Earth Policy Institute from U.N. Food and Agriculture Organization, Forest Resources Assessment 2010: Global Tables (Rome, 2010), www.fao.org/forestry/fra/fra2010/en/.

In the 2000s, around 30% of Earth's land area was covered by forest. More than 50% of forest is in the tropics and the rest is distributed across the boreal region, sub-tropics and temperate regions (Percy *et al*., 2003). From the 1990s to the 2000s, forest cover in the tropics declined by 14.2 million hectares, whilst non-tropical forests increased (FAO, 2001).

In Southeast Asia forest cover declined from 268.0 million hectares in the 1990s to 236.3 million hectares in 2010s (FAO, 2017; Stibig *et al*., 2014). The major cause of deforestation in Southeast Asia is agriculture expansion, which contributes to high biodiversity loss with the predicted extinction of 13 – 42 percent of terrestrial plant and animal species by the 2100s (FAO, 2017). Mining and urban development are also major threats to forest and biodiversity in South Asia, East Asia and Pacific (FAO, 2017). However, forest cover for the Asia-Pacific region as a whole actually increased from 731.1 million ha in 2000 to 734.2 m in 2005 at 0.09 percent of annual change rate, because of large reforestation campaigns in China (Figure 2.2) (FAO, 2005).

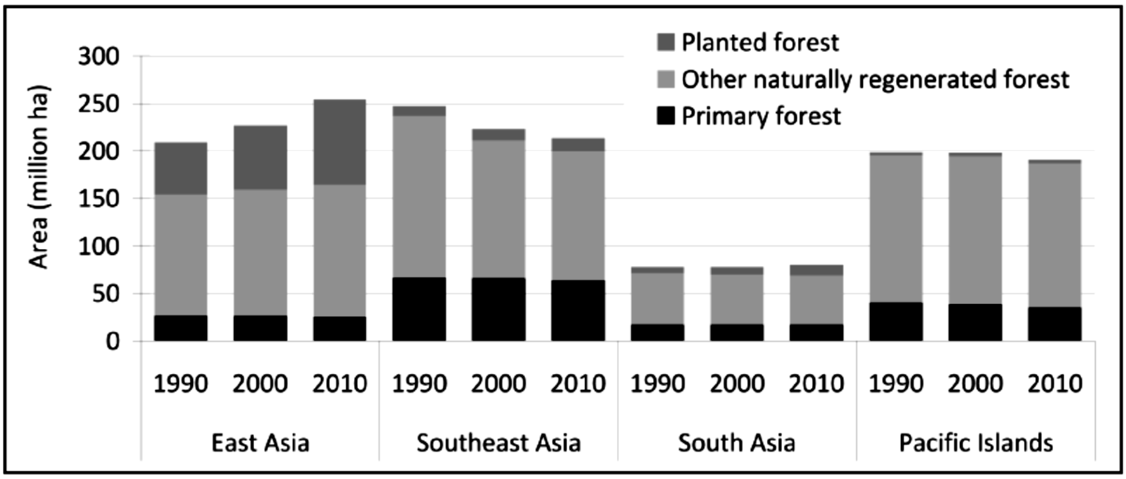


Figure 2.1 Category of forest area in Asia-Pacific sub region during 1990s to 2010s. (FAO, 2017; http://www.fao.org/asiapacific/forestry-outlook)

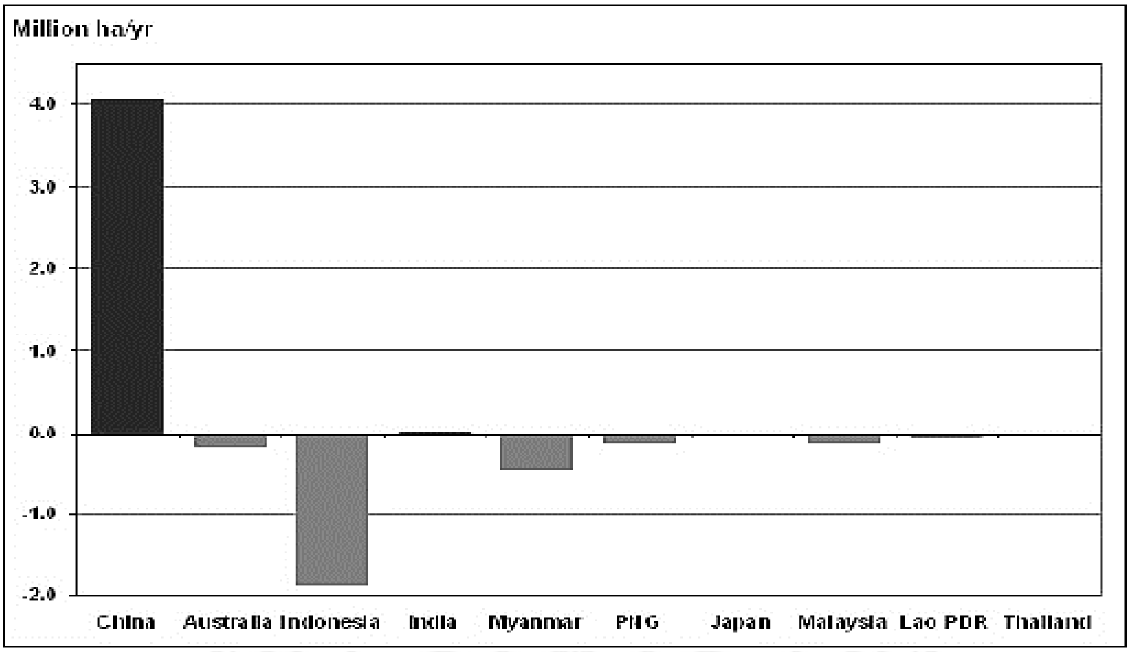


Figure 2.2 Annual change of forest area in ten largest forest area countries in Asia-Pecific (FAO, 2017; http://www.fao.org/docrep/011/i0627e/I0627E05.htm).

Focusing on Thailand, deforestation is one of main environmental issues. Thailand's forest cover declined from 53.33 percent to 25.13 percent of contry's total land area from 1961 to 1998 (Lakanavichian, 2001), appeared to increase suddenly to 32.66 percent in 2004 due to higher resolution satellite images being used to assess forest cover (RFD, 2004). The most rapid deforestation occurred from the late 1970s to early 1980s (Lakanavichian, 2006). In 2015, total forest cover was reported at 32.1 percent of total country area (Trading Economics, 2017). The main cause of forest destruction is agricultural expansion and logging. The logging and commercial timber product ban in 1989 helped to slowdown net forest change in Thailand (Lakanavichian, 2006).

Forest destruction negatively affects living organisms both directly and indirectly. Wildlife loss their habitats and provisions. It is estimated that more than 100 plant and animal species in tropical forests go extinct every day (Aerts and Honnay, 2011; Secretariat of the Convention on Biological Diversity, 2010). In addition, forests lose their ability to provide ecosystem services and forest ecosystem functioning, for example, decomposition of organic matter and water regulation (Aerts and Honnay, 2011; Duffy, 2009). Forest cover loss reduces rainfall in dry season (Delang, 2002). Runoff regulation declines, leading to more intense floods in the rainy season (Aerts and Honnay, 2011). Climate change and global warming are also included amongst the negative effects of deforestation (Stocker *et al*., 2013).

2.2 Forest restoration

Whilst deforestation is largely human-caused, forest recovery on degraded areas can be natural or human-assisted or managed (Lakanavichian, 2006). Natural forest recovery differs in pattern and dynamics, depending on the history and severity of disturbances (Breugel, 2007; Holl, 2012). Recovery of natural processes can be slow, because of limiting factors, such as lack of a seed bank, microclimatic conditions, soil degradation, competition with exotic grasses and herbaceous weeds, seed and seedling predation and lack of a soil seed bank of forest trees (Aide and Cavelier, 1994; Holl, 2012). So, forest restoration is an essential key to accelerate forest recovery (Aerts and Honnay, 2011).

The first step of any restoration project should be the identification of goals and specific objectives (Figure 2.3). Evaluation of the stage of degradation helps with plans to identify seed resources, and plan costs, labor and processes to support a successful restoration project (Holl, 2012). Normally, reforestation is measured in terms increases in biomass, structural complexity, biodiversity and ecological functioning. The main goal of forest restoration is to bring back a forest community where the aforementioned 4 parameters are similar the pre-disturbance condition (Fukami and Lee, 2006; Holl, 2012). The recovery of complex forest ecosystems leads increased biodiversity recovery and increased ecosystem functioning including carbon storage, nutrient cycles and watershed services (Palmer *et al*., 1997; Lamb *et al*., 2005; Gamfeldt *et al*. 2008; Isbell *et al*., 2011; Aerts and Honnay, 2011). Many organizations have achieved effective techniques to restore forests (i.e. International Tropical Timber Organization (ITTO) and IUCN) (Lamp *et al*., 2005). The restoration technique applied should be selected according to the severity of forest degradation (i.e. the level of degradation as in Elliott *et al*. 2013).

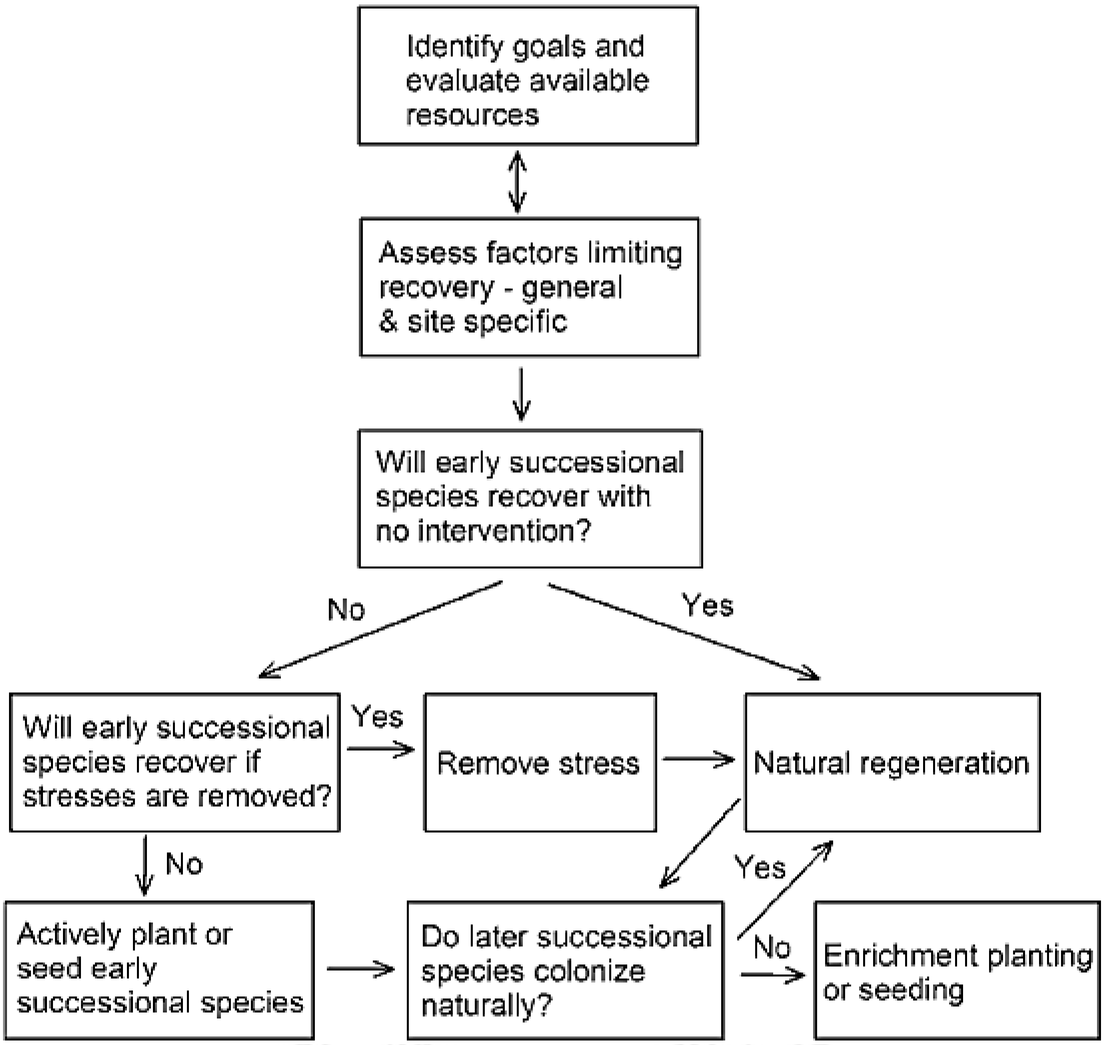


Figure 2.3 The selection process for tropical forest restoration techniques (Diagram from: Holl, 2012).

Forest restoration by planting indigenous tree species is recommended (FORRU 2006), especially those with broad dense crowns to shade out weeds and those which encourage seed dispersal by birds and improve the soil (Holl, 2012). Farwig *et al.* (2008) found that planting a mixture native tree species attracts birds and the species composition of the bird community in restored areas becomes similar to that in nearby natural forest. In contrast, bird species diversity in monocultures and exotic plantations is usually less than that in natural forest. Monocultures and exotic plantations support different bird species than natural forests do. To restore forest ecosystems, selecting mixtures of native tree species is recommended rather than exotic species. Forest restoration by planting non-native tree species contributes to new colonizing community, leading to change the original forest processes and ecological functioning and affects plant or animal specialist species in the areas (Magura *et al*., 2002)

The Miyawaki method of forest restoration originated in Japan in the 1970s and has been applied successfully in every region of Japan, South-East Asia, China and South America. This method is used when degradation is severe enough to prevent incoming seed dispersal. The process includes vegetation and soil survey and selects native tree species for planting at the high densities (Miyawaki, 2004). Degraded sites can be transformed into fully functioning forest in about 15-20 years using this method, which involves planting multiple tree species.

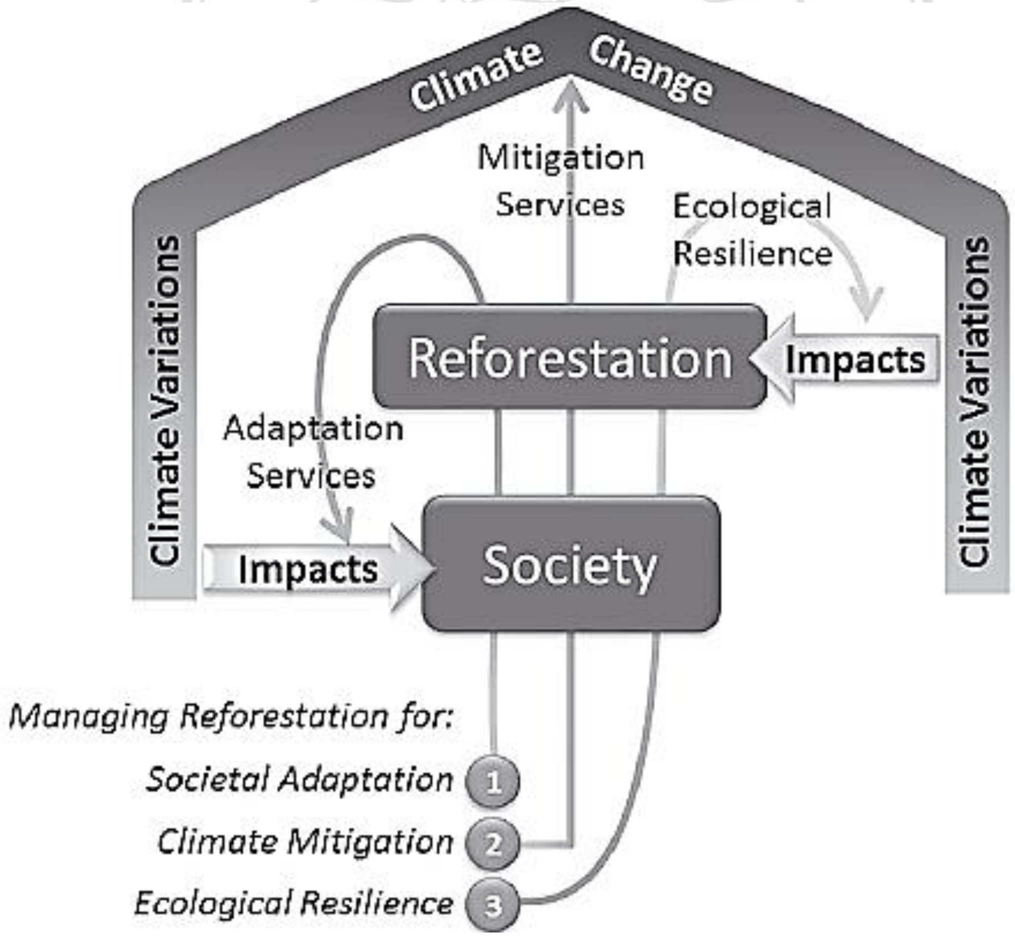


Figure 2.4 Climate-smart reforestation idea for forest restoration management (Locatelli *et al.*, 2015).

The framework species method is used to restore slightly less degraded sites where natural seed dispersal is still possible. It involves planting multiple indigenous forest tree species, including both climax and pioneer species to encourage rapid growth, shade out weeds and attract animal dispersers (Aerts and Honnay, 2011; Elliott *et al*., 2002). Reforestation by native species depends on species selection, site plan and management to attract dispersers and to reduce stresses conditions (Cunningham *et al*., 2015). The selection of native species following their functional group requires knowledge about traits, their reproductive biology, phenology and propagation (Thomas *et al*., 2014). Moreover, genetic variation and inbreeding between species in small population size should be considered for forest restoration by native species (Thomas *et al*., 2014).

Forest restoration can both mitigate global climate and is affected by it (Wright *et al*., 2009). Climate change affects tropical forest structure and their dynamics; for example, increasing temperature may affect biological processes in plants and plant-soil relations (Lewis *et al*., 2004). Moreover, reduced precipitation, resulted from climate change, limits plant growth and forest regeneration (Lewis *et al*., 2004). So, climate-smart reforestation should be encouraged at the aim for forest migration and adaptation in climate situation and future direction (Locatelli *et al*., 2015). Species selection for reforestation should be high resilient and can adapt to climate change situation (Figure 2.4).

Forest restoration is time-consuming and expensive. Before planting trees, seeds must collected usually from natural forest. Seeds are germinated and seedlings raised in tree nurseries. (Lamb *et al*., 2005; FORRU, 2006; Bruel *et al*. 2010). Seedling production requires building and maintaining a tree nursery. In addition, seedlings in nurseries require constant care by highly skilled nursery staff (FORRU, 2006). Therefore, the construction, maintenance and labor costs of conventional tree planting are costly and time-consuming (FORRU, 2006; Bruel *et al*. 2010).

2.3 Direct seeding

Direct seeding involves sowing seeds directly into the substrate of restoration sites (Ochsner, 2001; NRCS, 2009, Birkedal, 2010). This method is commonly used to grow most annual crops (Balasubramanian and Hill, 2000) and more rarely to promote biodiversity recovery in natural forests and for reclamation of limestone mines (Kumar and Ladha, 2013; Hossain *et al*., 2014). Direct seeding has been successfully used to restore broadleaved woodland (Willoughby *et al.*, 2004), coniferous forests (Nilson and Hjältén, 2003), Beech and Oak forests (Birkedal, 2010), pasture land (Douglas *et al*., 2007) and limestone mines (Barton *et al*., 2015).

Direct seeding can result in trees with higher performance than those from conventional tree planting (Tunjai, 2005; NRCS, 2009). Seedlings from direct seeding are stronger, taller, more robust, have broader crowns and higher survival rates compared with planted nursery-raised seedlings (Tunjai, 2005; NRCS, 2009). The method is about 20 – 50 percent cheaper than tree-planting (Willoughby *et al*., 2004; Birkedal, 2010). There are no nursery costs and transporting seeds is easier than seedlings (Birkedal, 2010; Farlee, 2013). However, weed removal costs may be higher for the direct seeding to ensure survival of the very small seedlings just after germination (Tunjai, 2011).

Douglas *et al*. (2007) suggested that appropriate tree species for direct seeding in pasture land are (1) native, (2) adaptable, (3) with wide environmental tolerance, (4) highly competitive with grasses, (5) with high germination and growth rates and (6) suited to the soil microbial status. It is necessary to select characteristic of tree species to increase the probability of seedling establishment (Lamb, 2005). Rapid seed .germination is preferable to minimize seed predation (Lamb *et al.*, 2005; FORRU, 2006; Tunjai and Elliott, 2011). Moreover, species should have dense spreading crowns to shade out weeds and provide resources, such as flowers and fruits, early in life to attract seed-dispersing animals (FORRU, 2006). In addition, seed traits are important because some are related to seedlings survival e.g. seed size, shape and moisture content all affect seedling establishment (Tunjai and Elliott, 2012). Previous studies show that large-seeded species have higher establishment rates than small seeded species (Doust *et al*., 2008, Tunjai and Elliott, 2011).

Site preparation is also important for the success of direct seeding (Douglas *et al.*, 2007). Weeds must be removed before sowing to reduce competition (Ochsner, 2001) and to reduce the habitat for seed or seedling predators (Birkedal, 2010). Site preparation can be done by mechanical treatments (Birkedal, 2010). Ploughing and herbicide spraying are options for weed control (Aleksandrowicz-Trzcińska *et al.*, 2014; Doust *et al*., 2006; Ochsner, 2001). Although herbicide is effective for weed control, its is not very practical since it kill natural regeneration, and affect environment and human (FORRU, 2006). Burning is not recommend because fires can destroy natural regenerants in the sites.

In some cases, soil testing should be done to determine nutrient levels. Soil manipulation helps to provide suitable microhabitats for seed sowing and to provide better conditions for direct seeding (Doust *et al*., 2006).

In general, seeds are usually collected from mother trees in natural habitat (Willoughby *et al*., 2004; FORRU, 2006; Doust *et al*., 2008). To restore degraded areas, local tree species from forest nearby the degraded site are selected. The Forest Restoration Research Unit (FORRU-Chiang Mai University) recommends phenology studies (time for flowering, fruiting and leafing) to determine the optimal seed collection time and to understand the ecological status of tree species in their natural habitats. Genetic variability should be also maximized by collecting seeds from many parent trees (FORRU, 2006; Doust *et al.*, 2008).

Seed storage behavior can also be important if direct seeding is carried out outside the fruiting period of the species being planted. The practical dimension to seed storage behavior contrasting patterns belong to (1) the effect of desiccation on viability and (2) seed longevity response to the storage condition (Hong and Ellis, 1996) (Figure 2.5). Furthermore, seed behaviors can also be predicted by seed coat ratio (SCR), using the proportion of dry seed coat and dry seed mass. Large seeds with low seed coat ratio tend to be recalcitrant and sensitive to dry conditions (Dawns *et al*., 2006). Three categories of seed storage behavior include orthodox, intermediate and recalcitrant.

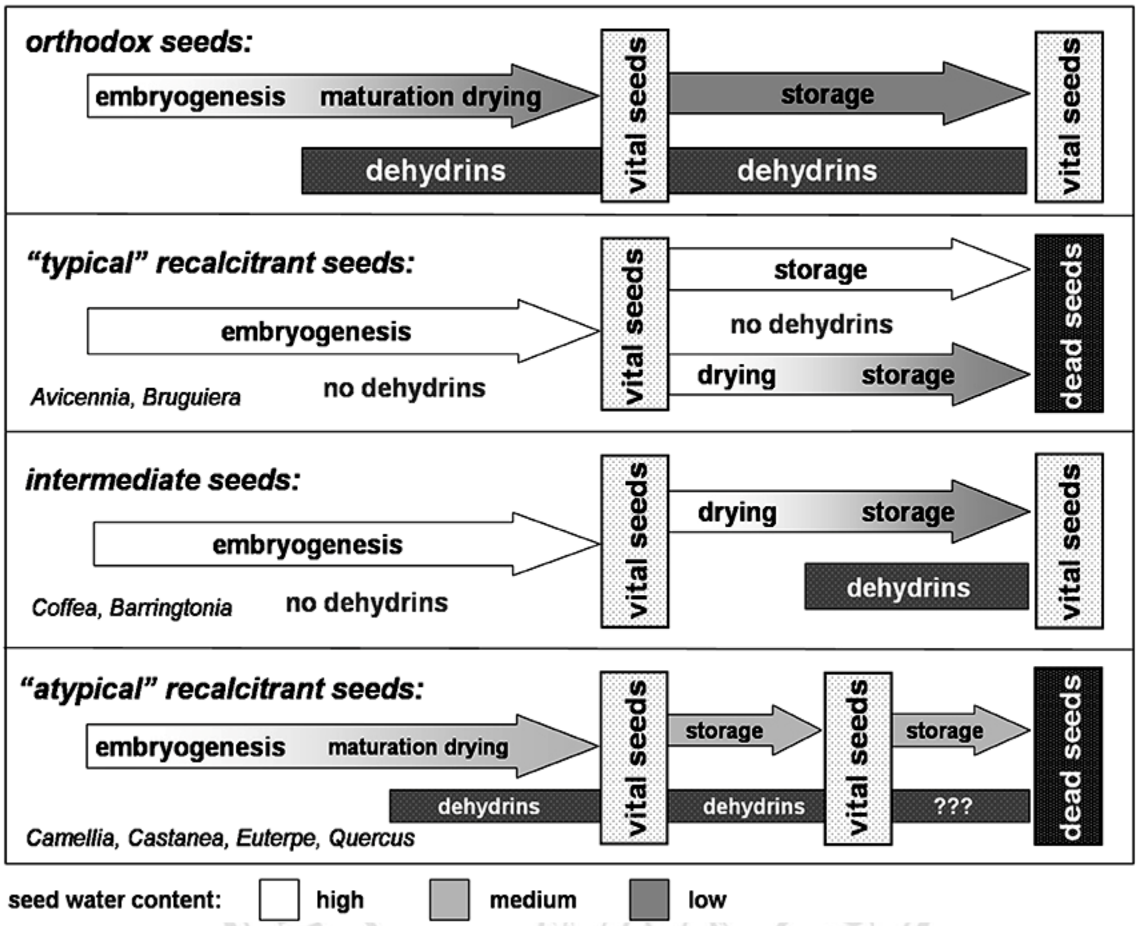


Figure 2.5 The dehydrin expression and maturation drying–an adjustment to the chain of seed behavior events (Radwan *et al.*, 2014).

Orthodox seeds can be stored in dry condition much longer than recalcitrant seeds can (Mag'omba, *et al*., 2007). The seed longevity increases with decrease in moisture content and temperature of seed storage in a quantifiable and predictable way (Radwan *et al.*, 2014; Roberts, 1973) (Figure 2.5). The germination will be happened in fully hydrated, this can be prevented by storage seeds in dormant condition (Hong and Ellis, 1996). However, if need to use some long dormancy-orthodox seed immediately after collecting from mother trees, seed pretreatments can be used to shorten the dormancy period and increase percent germination (Willoughby *et al*., 2004). Effective pretreatments vary among tree species, depending on seed characteristics. For example, seed testa removal increased germination of *Uapaca kirkiana*seeds to 100% (Mag'omba, *et al*., 2007).

Recalcitrant seeds cannot be dried without damage (Roberts, 1973). Seed viability losses in dry seed storage environments, which reduced seed moisture content (Hong and Ellis, 1996) (Figure 2.5). There is still no method to preserve the viability of recalcitrant seeds over long term due to they cannot be dried and are sensitive to subzero temperature (Hong and Ellis, 1996). This means that recalcitrant seeds can only be used for direct seeding during or very shortly after the seed are collected from the mother trees (Mag'omba, *et al*., 2007). Nevertheless, short-term storage under specialized conditions is possible for recalcitrant seeds (Hong and Ellis, 1996).

Intermediate species have seed strong behaviors between those of recalcitrant and orthodox species (Figure 2.5). The intermediate category was subdivided and introduced more recently to complete the loosely gap in classification between recalcitrant and orthodox categories (Hong and Ellis, 1996). These seeds are known to have high water content level and tolerance to dehydration (Mag'omba *et al*., 2007). Intermediate seed may be appropriate for direct seeding.

Sowing time influences seedling establishment. For example, Doust *et al*. (2008) reported higher seedling establishment for seeds sown late in the wet season (Doust *et al*., 2008). Seedlings from seeds that were sown in early rainy season had lower development root systems (Doust *et al*., 2008). However, another study suggested different sowing time. Brikedal, 2010 reported that sowing in early rainy season enabled better root system because of longer time to grow. Weed competition is an important limitation when implementing early sowing, whereas water supply is limiting for late sowing in rainy season (Doust *et al*., 2008).

Additionally, site preparation and intensive site maintenance contribute to increased seeding growth and deeper root systems (Lof and Birkedal, 2009). Seeds should be sown two weeks after weed removal by herbicide for site preparation. Small-seeded species are usually sown at higher densities than large-seeded species (Doust *et al*., 2008). Burying seeds at an appropriate depth reduces seed predation (Doust *et al*., 2006, Farlee, 2013). In addition, weeding is usually done two months after direct seeding to reduce competition with herbaceous weeds (Doust *et al*., 2008; St-Denis *et al*., 2013). Fertilizer is applied after weeding. Both weed control and fertilizer application are needed for site maintenance for at least three rainy seasons in tropical areas before the trees can begin to close canopy and shade out weeds (FORRU, 2006).

2.4 Limitations/failures of direct seeding

Direct seeding can often be more successful than conventional tree planting (Lamb, 2005), but there are challenges in wet tropical environments (Holl *et al.*, 2000), such as environment conditions (Douglas *et al*., 2007), competition with herbaceous weeeeds (Douglas *et al*., 2007; Doust *et al*. 2008) and seed and seedling predation (Fricke *et al*., 2014; Hau, 1997; Orrock *et al*., 2006).

This study focuses on effect of natural enemies on seeds and seedlings. Seeds can be killed when animal predators completely consume or partially damage the seeds (Janzen, 1970). The destruction of seeds leads to low seed availability and loss of germination and/or growing ability (FORRU, 2006). For seedlings, being completely or partially consumed by animals lead to loss of growing ability and death. Consequently, attacks by seed and seedling predators may lead to failure of forest restoration by direct seeding method (Farlee, 2013).

2.5 Seed removal and seed predation

Seeds may be removed by secondary seed dispersers and/or predators. If seeds are removed by secondary seed dispersers, they are not killed but are transported to new areas. Seed predation is the consumption or destruction of seeds by granivorous animals (Vander Wall *et al*., 2005). Seed predation usually occurs on the ground (Vander Wall *et al*., 2005). In the direct seeding context, seed removal from the target area reduces the number of seeds available for seedling establishment on the restored site. In this study, seed removal is used as a proxy to estimate seed predation.

The major group of invertebrate predators is insects, including beetles (Coleoptera), ants and wasps (Hymenoptera), flies (Diptera), caterpillars of butterflies and moths (Lepidoptera), and thrips (Thysanoptera) (Zhang *et al*, 1998). In some degraded areas, ants are major seed predators (Wood and Elliott, 2003). Of the vertebrates, mammals, such as rodents, are most commonly associated with seed predation and seed loss (Birkedal *et al*., 2010; Wood and Elliott, 2003). Large seeds are lost to rodents but small seeds are not destroyed. At the post-dispersal stage, both vertebrates and invertebrates are major seed predators.

The intensity of seed predation varies, according to the predator communities that are present in different forests types or degraded areas (Wells and Bagchi, 2005), which is related to availability of food resources (Doust *et al*., 2006). Seed predation have been recorded high rate in open woodland area (Nilsson *et al*. 1996; Farlee, 2013). In degraded grassland and shrub lands in Hong Kong, a high percent seeds are lost (11 from 12 seeds species were completely removed) due to predation by rats (Hau, 1997). In contrast, seed predation occurs at lower seed removal in abandoned agricultural lands in northern Thailand (Woods and Elliott, 2003).

The intensity of seed removal and seed predation depends on predators' body sizes relative to seed size (Wells and Bagchi, 2005). Small-seeded species suffer less predation than bigger seeded species (Ferreira *et al*., 2011). In addition, the relationship between seed size and predation rate also depends on habitat type, the searching ability of seed predators and whether seeds are on the soil surface or buried (Moles and Westoby, 2006). Seeds with soft seed coats are significantly more attractive to seed predators on degraded hillside than those with harder seed coats (Hau, 1997). Therefore, the intensity of seed predation depends on a combination of many factors that should be considered case by case.

2.7 Seedling predation (Herbivory)

In tropical forests, the majority of damage by herbivores occurs on young leaves (Kursar and Coley, 2002). Young leaves are attractive to herbivores, because they lack structural carbohydrates, which make the leaves tough and less digestible (Coley, 1983). Seedlings have a low investment in defensive chemical because of limited photosynthetic ability and root biomass (Boege and Marquis, 2005). Herbivory reduces seedling growth and survival, their competitive ability against weeds (Mills, 1983).

After germination, seedlings are usually attacked by invertebrates, particularly insects (Doust *et al*., 2008; Fricke *et al*., 2014). Total plant biomass is mostly reduced more by invertebrates than by vertebrates (Gurevitch *et al.*, 1992; Meiners *et al*., 2000). Invertebrates attack both above- and below-ground plant parts. Leaf-feeding herbivores affect plant growth by reducing photosynthetic capacity and by decreasing carbohydrate reserves (Wahungu *et al*., 2002). In the case of sap-feeding insects, they can kill seedlings without obvious damage to the leaves and/or stems (Meiners *et al*., 2000). Insects can also heavily damage germinating seeds and young seedlings below ground (Meiners *et al.*, 2000)

Herbivory by small mammals also affects seedling survival and establishment (Birkedal *et al*., 2010; Wahungu *et al*., 2002). Small mammals (rodents) can significantly reduce seedling survival (Zhang *et al*., 2017). Rodents can kill seedlings by clipping their shoots and removing their cotyledons. In direct seeding trials, few studies have been done on seedling predation by vertebrates (Birkedal *et al*., 2010). The effects of rodents on seedling survival may be reduced by site preparation and management (Birkedal *et al*., 2010).

2.8 Methods used for studying predators

Many studies of post-dispersal seed predation published in natural forests (Cramer *et al*., 2007; Ferreira *et al*. 2011; Wahungu *et al*., 2002), grasslands (Bricker *et al*., 2010; Pufal and Klein, 2013) degraded forests (Hautier *et al*., 2010), agriculture lands and abandoned agricultural areas (Rocha-Ortega *et al*. 2016; Pufal and Klein, 2013; Wood and Elliott, 2003). One way to determine the intensity of seed and seedling predation is to exclude predators from sample plots and then compare seed loss with control plots exposed to predators. For example, Fricke *et al*. (2014) studied the effects of natural enemies on tree survival and density-dependent mortality. The experiments included an insecticide treatment to exclude insects, fungicidal treatment to prevent fungal infection and enclosure to protect seeds from small mammals (Fricke *et al*., 2014). The experiments allowed comparisons among treatments, to determine the cause of density-dependent mortality. Effect of pesticide (fungicide or insecticide) on seedling germination, survival and growth usually test on crop plants (e.g. Onemli, 2004; Udaiyan et al., 2001). A few work was done in tree seedling. For example, Rolando (2006) claim that insecticide supported survival of pine species during regeneration period. However, the violence of insecticide depends on chemical types, concentration and plant species treat by insecticide (Robinson, 1985)

Knowing the species of seed and seedling predators helps in managing sites to prevent predation (Birkedal, 2010). Animal surveys are the primary steps used to identify species and their roles in plant-animal interactions. Different groups of animals require different survey methods.

Camera trapping has been widely used for monitoring wildlife diversity, activity patterns and population dynamics. It is also a standard sampling technique for some rare species (McDonald *et al*., 2015). Camera trapping has been effective in determining abundance of animals and their activity patterns in nature reserve (Liu *et al*., 2013). Kukielka *et al*. (2013) successfully used camera traps to monitor interaction between wildlife and livestock at water bodies during the dry season. In Central Panama, Meyer *et al*. (2015) used camera traps to estimate species richness, evenness and community structure of forest mammals. Camera traps have been used to detect medium to large animals, as well as small animals including rodents (De Bondi *et al*., 2010; McDonald *et al.*, 2015; Melidonis and Peter, 2015). One advantage of the technique is that of animals can be observed continuously, allowing more accurate estimates of animal abundance. De Bondi *et al*. (2010) surveyed small mammals by live trapping and compared abundance estimated that obtained with camera trapping. Camera trapping recorded more animal species than live trapping did. Camera traps can continue working long periods and are effective at capturing undisturbed animal activities.

However, camera trapping is not suitable for some species (Pollock *et al*., 2002). Camera sensors can detect animals by motion when they are passing the detection zone. In addition, camera sensors detect differences between body temperature and ambient temperature (Rovero *et al*., 2013). If an animal has similar body temperature as the environment such as reptiles and amphibians, the cameras may not be triggered animals.

Camera trapping is not a good method for insect surveying. Insects are too small to trigger motion sensor and their body temperature is similar to ambient temperature. Other insect sampling method include netting (sweep net method), flight intercept trapping, pitfall trapping, light trapping, sticky trapping, etc. (Upton and Mantle, 2010).

Sticky traps used to collect insects in forest and farmland (Atakan and Canhilal, 2004). They have been used in agricultural lands to estimate insect pest (Silvanderson, 2015) and for studies the population dynamics of parasitoids on crop plants (Qiu and Ren, 2006). However, sticky trap mostly captures abundant flying insect species. Whereas studies of ground-dwelling insects mostly use pitfall traps (Upton and Mantle, 2010). Such trap are constructed by placing a plastic cup into the soil with alcohols or detergent and protecting from rain by a cover (Gadagkar *et al*., 1990). Therefore, different traps have different effective to collect insect group. The target insect group should be considered before trapping selection.

CHAPTER 3

Methods

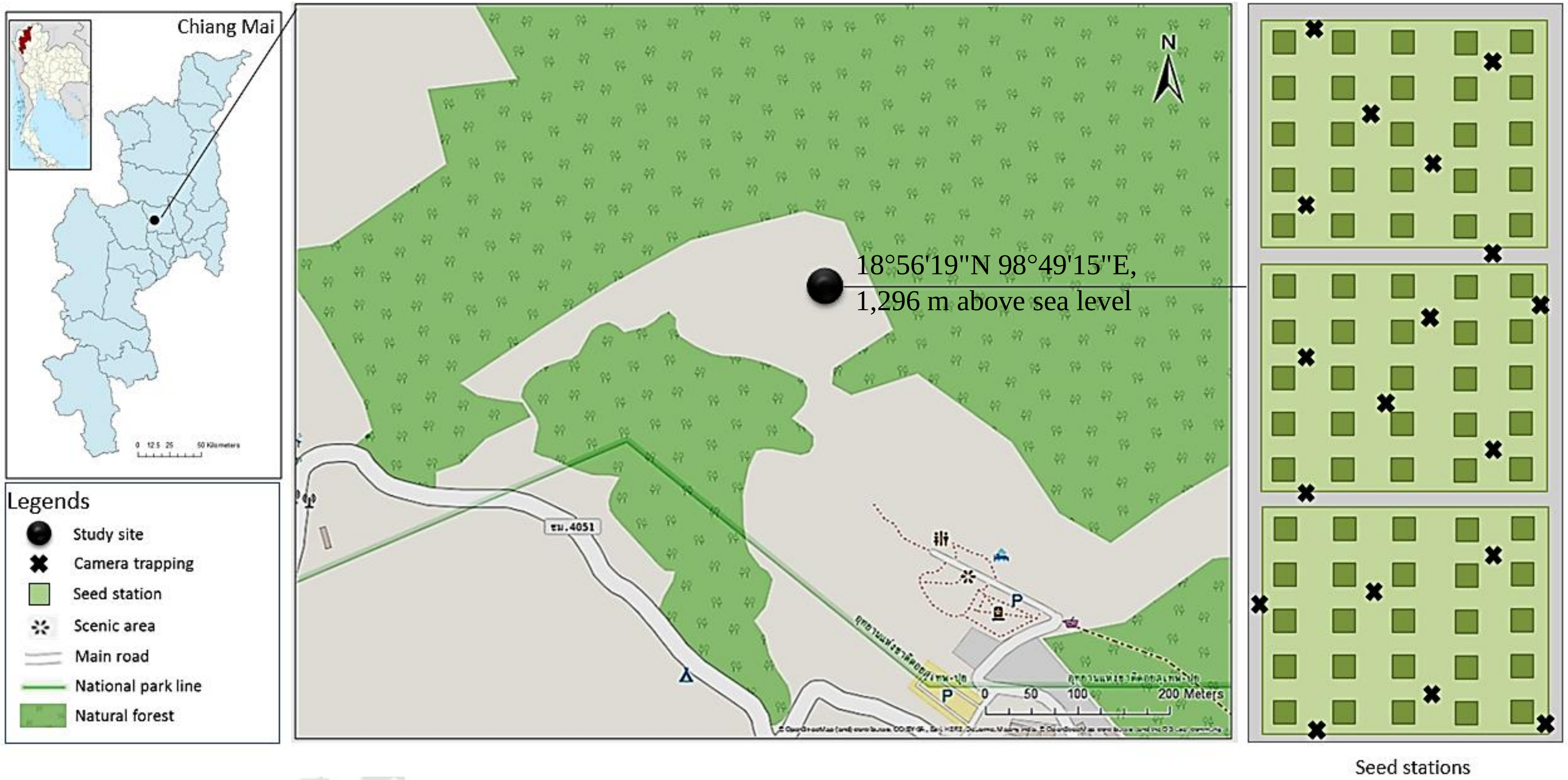
3.1 Study site

Field site

The study site was at Nong Hoi Royal Project Foundation (Mon-Cham) degraded area (18°56'19''N 98°49'15''E, at about 1,296 m above sea level), in Doi Suthep-Pui National Park, Chiang Mai, Northern of Thailand (Figure 3.1). The average annual precipitation from January 2015 to December 2016 was 1,419 mm with a dry season from December to April. The average annual temperature and humidity were 22ºC and 75.4%, respectively (Figure 3.2). The study site was 650 m^2 in area and 70 m away from the nearest natural forest. The degraded area was previously used as agricultural land with intensive chemicals for growing crops such as cabbage. The ground herbaceous plants were dominated by Bracken fern (*Pteridium aquilinum*), Cogon grass (*Imperata cylindrical*) and Green panic grass (*Panicum maximum*) (Figure 3.3). Recently this area was reserved for forest restoration by the Royal Project in 2012 with technical guidance from FORRU-CMU[1].

[1] Chiang Mai University's Forest Restoration Research Unit

Figure 3.1 The location of study site (black circle) Mon-Cham degraded area in Ban Nong Hoi, Mae Rim, Chiang Mai, Thailand (inset map top left)



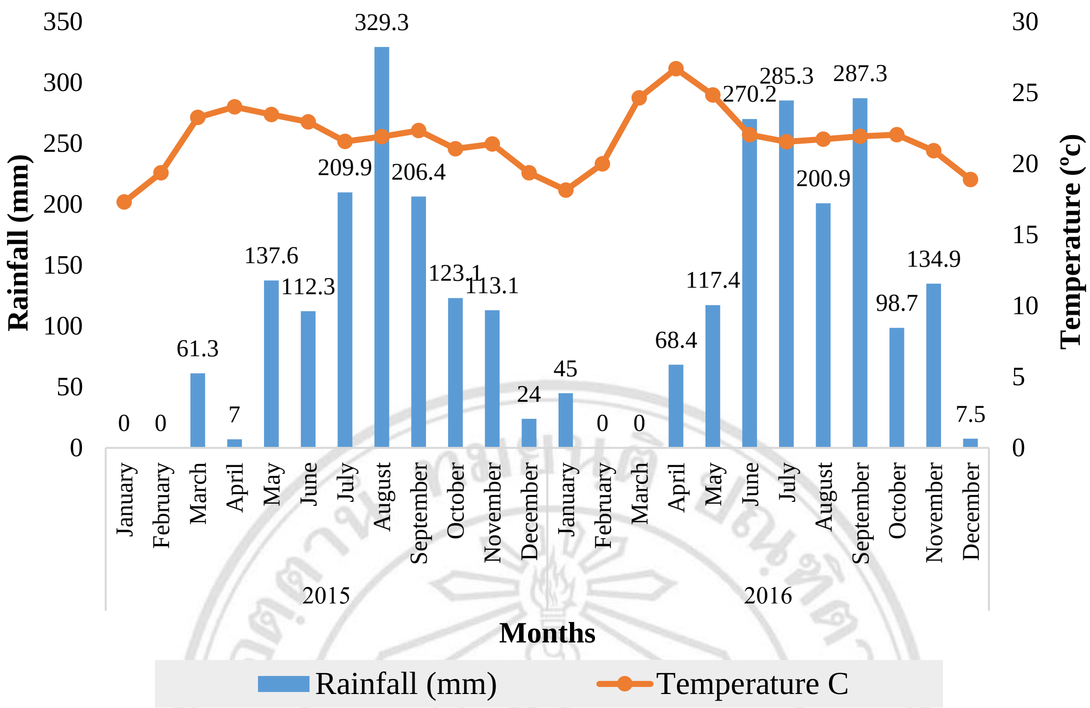


Figure 3.2 Climatic data of Ban Nong Hoi (Mon Cham) from January 2015 to December 2016. Graph showed amount of rainfall precipitation (▬, mm per month) and average temperature in each month (-●-, ºC) (Meteorological Department of Thailand, 2015).

Figure 3.3 Study site at Mon Cham, Mae Rim District, Chiang Mai province.

The nursery

Seed germination tests for this study were conducted at FORRU's research nursery (Figure 3.4), located in Doi Suthep-Pui National Park (18°48'3.7" N 98°54'59.6" E, at about 1,000 meters from sea level). Tree seedlings were looked after and watered by FORRU's staff.

Figure 3.4 the FORRU's nursery in Doi Suthep-Pui National Park

3.2 Tree species studied

Five tree species were selected for experiments: *Hovenia dulcis*, *Alangium kurzii*, *Prunus cerasoides*, *Choerospondias axillaris* and *Horsfieldia glabra* (more details about these species are provided in Appendix A). All studied species were native to hill evergreen forest near the field study site (above 1,000 meter in elevation) (FORRU, 2006) in Doi Suthep-Pui National Park, Chiang Mai, Northern of Thailand (Table 3.1 and Figure 3.4).

Propagules, either seeds or (in the case of *Prunus cerasoides* and *Choerospondias axillaris*) pyrenes were collected early in the rainy season, from May to July 2015 (Table 3.1). At least 600 propagules were collected from five mother trees of each species, mixed, cleaned, dried, and stored at room temperature, until they were used in experiments.

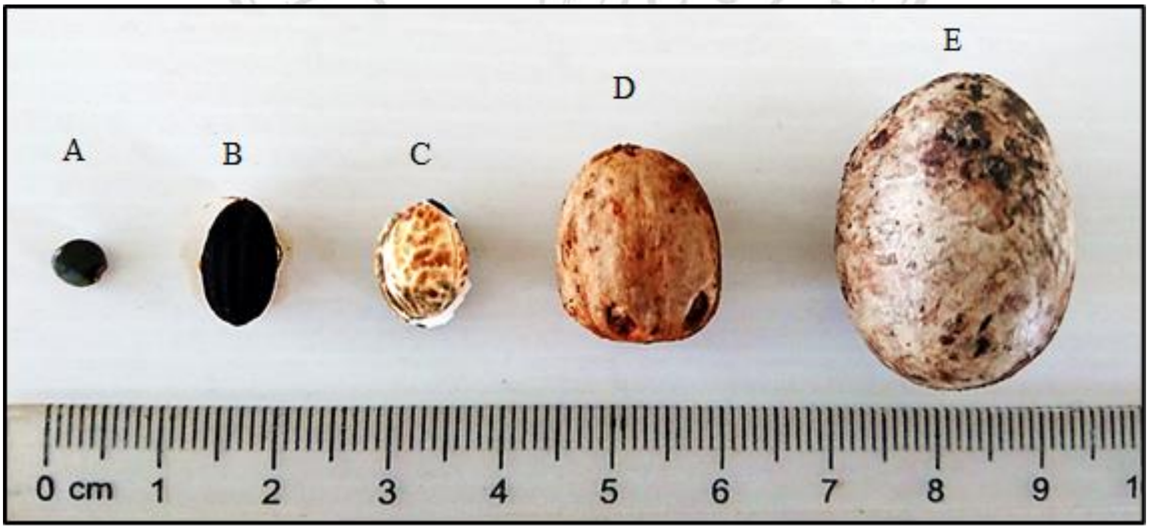


Figure 3.5 Propagules of five tree species from smallest to the largest; *Hovenia dulcis* (A), *Alangium kurzii* (B), *Prunus cerasoides* (C), *Choerospondias axillaris* (D) and *Horsfieldia glabra* (E).

Table 3.1 Information about the five native tree species were used in this study

Species name	Seed behavior	Seed (propagule) type	Seed dry weight (g)	Seed volume (mm^3)	*Germination rate
Hovenia dulcis (RHAMNACEAE)	Orthodox	Hard-two layer of seed coat with 1 seed/locule	0.024 ± 0.000	47.38 ± 1.34	60-70%
Alangium kurzii (CORNACEAE)	Orthodox	Pyrene with 1 seed/locule	0.179 ± 0.027	392.27 ± 11.22	10-92%
Prunus cerasoides (ROSACEAE)	Orthodox	Pyrene with 1 seed/locule	0.287 ± 0.034	424.94 ± 7.89	76%
Choerospondias axillaris (ANACARDIACEAE)	Orthodox	Pyrene with 5 seeds/locules	2.602 ± 0.320	3575.19 ± 125.16	43%
Horsfieldia glabra (MYRISTICACEAE)	Recalcitrant	Soft seed coat with 1 seeds/locule	4.247 ± 0.664	7507.25 ± 322.89	94%

*Data from FORRU (2006)

3.3 Predator exclusion experiments

3.3.1 Experimental plots and predator exclusion experiments

To determine the effects of seed predators on seed removal and seed germination, I used predator exclusion experiments (Figure 3.6 and Figure 3.7). There were five treatments: -

T1: Wire cage, to protect seeds and seedlings from vertebrates (CA),

T2: Insecticide only, to protect seeds and seedlings from invertebrates (IN),

T3: Wire cage plus insecticide, to protect seeds and seedlings from vertebrates and invertebrates (IC),

T4: Open cage to control for the presence of cage (OC), and

T5: Control with no protection, no treatment applied and exposed to invertebrates and vertebrates (CO).

Figure 3.6 The exclusion experiments were established in the study site.

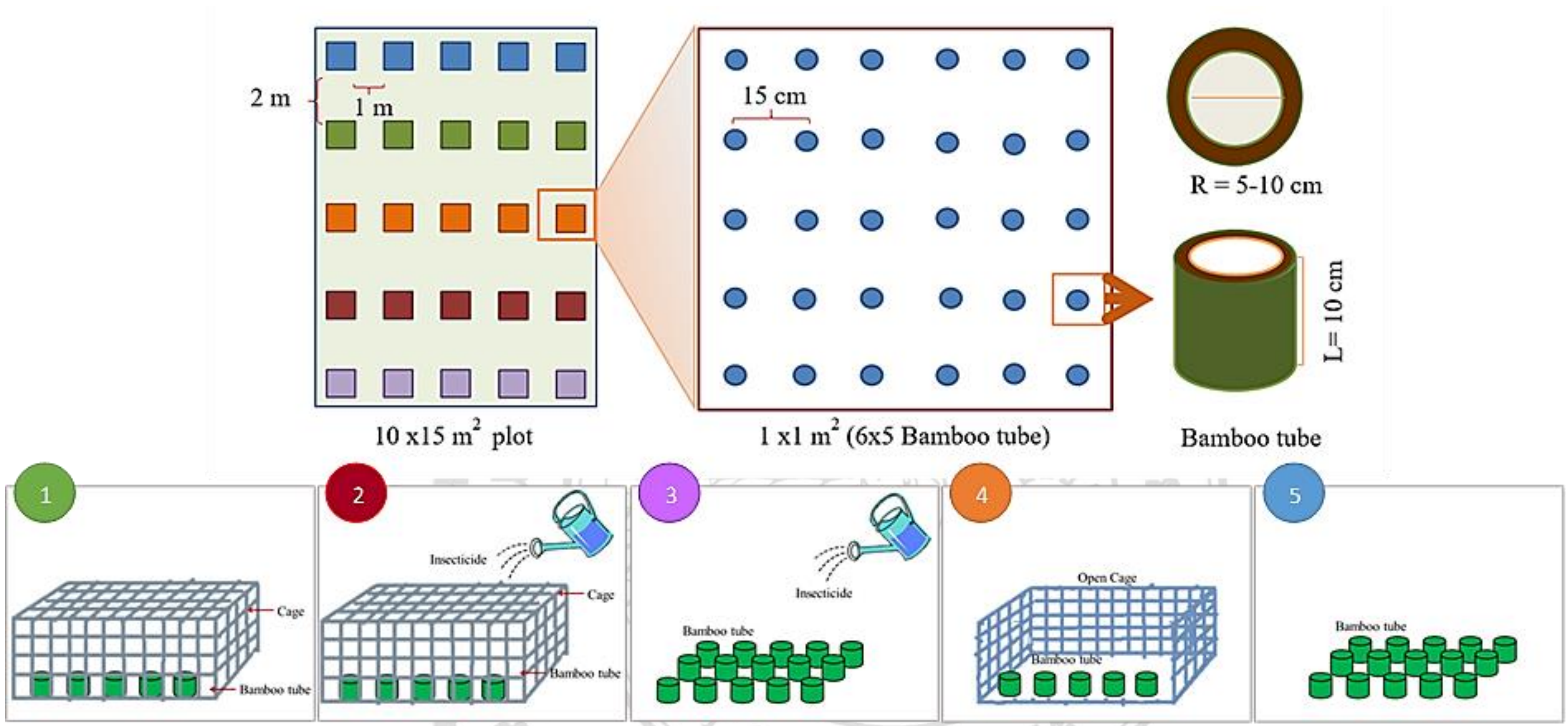


Figure 3.7 Arrangement of predator exclusion experiments in one replicate of 10 x 15 m^2 plot. There were 30 bamboo tubes in each subplot (1 x 1 m^2). One seed was sown in each bamboo tube in each subplot. Below, the plot arrangement for the five predator exclusion treatments.

Cages were built using bamboos and steel wire. The open cage was made in the same fashion as the wire cage but the top and one lateral side of the cage was removed. The insecticides Chlorpyrifos (Trade name: Kino505: Appendix D) were spayed every week from seed sowing in July until December, after all seedlings had emerged. The insecticide was mixed with water in the ratio of 2.5 ml of insecticide per 1 liter of water in a pressure spray. For each species, the 200 ml of the insecticide mixture was sprayed homogenously onto each replicate of the insecticide and the insecticide plus cage treatment.

At the Mon-Cham site, three 10 x 15 m^2 experimental plots were established in July 2015 in the middle of rainy season. The three plots were about 5 m from each other and each plot (a block) contained one of each treatment. Each plot was divided into five rows, two m apart, to accommodate five treatments. In each row, five 1 x 1 m^2 sub-plots were established to accommodate the 5 species studied. In each sub-plot, 30 bamboo tubes were buried to 5 cm deep into the soil (Figure 3.7). In each tube, one seed was sown on the soil about 1 cm deep and covered by soil (all seeds were buried).

Two treatments are applied during the nursery experiments: with and without insecticide (the same as in the field experiment). To examine whether the insecticide inhibited seed germination, seeds of each tree species were sown in germination trays and covered by soil. Ninety seeds were sown per tray per treatment (two trays per species). Each treatment was placed far away from each other, to avoid possible insecticide drift on to the control. Germinating seeds were counted weekly and the percent germinant was compared between the two treatments (Figure 3.8).

Figure 3.8 Germination tray with seeds (1 module per seed) in nursery experiment.

3.3.2 Survey of potential seed predators

To identify potential predators, I collected the data on small mammals, birds and insects visiting the experimental plots.

Vertebrate species - small mammals and birds

Small mammals and birds were surveyed, using five camera traps for seven months from August 2015 to February 2016. The occurrence of vertebrate species was captured using RECONYX™ PC900 HyperFire™ cameras (set for five snapshots per detection). Each camera trap was mounted in a plastic case and attached to an iron bar, 40 cm above the ground (Figure 3.9). The camera traps were randomly placed across the site (Figure 3.1) - over seven months, a total of 28 trapping locations.

Small mammals and birds, detected by the camera traps, were identified to species or genus. We used “A Naturalist’s Guide to the Mammals of Thailand and Southeast Asia” (Shepherd and Shepherd, 2012) and “Guide to the Birds of Thailand by Boonsong Lekhakul” (Nabhitabhata *et al*., 2012) for species identification. In addition, the time and date, the number of photos and the number of individuals were recorded and counted from the photographs; whenever a single species appeared in photographs taken more than 30 minutes apart, the two subjects were treated as separate individuals (O’Brien et al., 2003).

Invertebrate species - insects

To collect insect specimens, pit-fall traps, sticky traps and direct collecting were used. Insect collections were done three times, (1) in August (after sowing, but before most seeds had germinated), (2) in October (after the small-seedling stage at the end of the rainy season), and (3) in April (with larger seedlings in dry season).

Pitfall traps were randomly installed near seed or seedling stations. Fifteen plastic cups, filled with a mixture of water and liquid detergent (100 ml water: 1 ml detergent), were randomly installed at 5 points per replicate (5 x 3 replicates) for three days (Figure 3.10A). The cups were placed in a small hole on the ground, so that the cup opening was at the same level of the soil surface. A piece of small plastic sheet (size: 5 x 5 cm) was placed to prevent additional accumulation of rain water, over of each pit-fall trap.

Fifteen sticky traps (size: 15 x 15 cm^2), made from yellow corrugated plastic with glue for insect trapping, were installed next to pitfall traps 10 cm above the ground (using 20 cm bamboo sticks as poles) (Figure 3.10B). Moreover, at the time of censusing seed removal and germination, insects that were found in the experimental plots were collected by hand.

Insect specimens were preserved in 75% alcohol and classified to Order and Family, according to their morphological characteristics using “An Introduction to the Study of Insects, 6th Edition” (Borror *et al.*, 1989).

Figure 3.9 Camera trap installed at the field experimental site

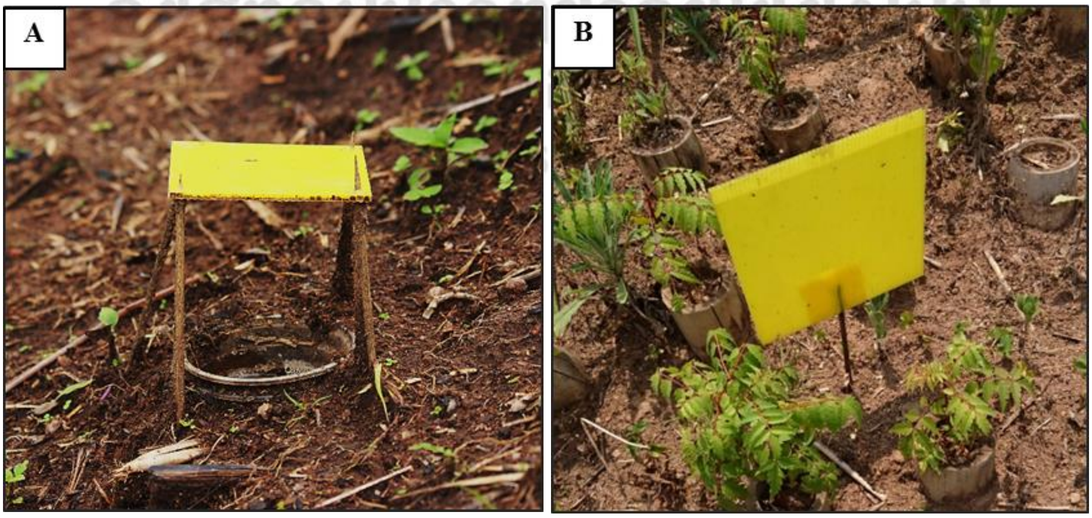


Figure 3.10 Pit-fall trap (A) and the sticky trap (B) in the study site.

3.4 Data collection and analysis

3.4.1 Seed removal and germination

The numbers of seeds removed from the bamboo tubes were counted weekly. Traces of seed removal were recorded, including scratches, digging marks and nests of some insects nearby the bamboo tubes. Germinated seeds were recorded as the presence of radicles and/or hypocotyl.

A generalized linear model (GLM) with a binomial family test in the R program ver. 3.4.1 was used to determine the effects of treatments on seed removal and germination. Species and treatments were used as independent variables. The dependent variable was the number of seeds removed or germinated.

Formula of GLM equation;

$$\mathbf{g(P) = ln(\frac{P}{P-1}) + \beta_0 + \beta_1 * Species + \beta_2 * Treatment + error}$$

In addition to seed germination in nursery, survival analysis and log-rank test (Chi-square test, *df* = 1, Critical value = 3.84) was used to determine the difference between control and insecticide treatments. Furthermore, seed germination in the field and the nursery were also compared by survival analysis and log-rank test (Chi-square test, df = 1, Critical value = 3.84).

3.4.2 Cotyledon-seedling and leafy-seedling mortality

In this study, seedling mortality was used as an index to estimate seedling predation. Germinants were classified as cotyledonous-seedlings if they possessed expanded cotyledons with no true leaves. Later, they were classified as leafy-seedlings once at least the first pair of true leaves had fully expanded (Figure 3.11). The numbers of cotyledonous-seedlings and leafy-seedlings that were dead were recorded. In addition, physical signs of damage to the dead seedlings that remained on-site were recorded and classified into two categories: damage by insects and wilting. Dead seedlings that had disappeared from the site were classified as “unknown cause of death”.

Percent mortality of cotyledonous-seedlings and leafy-seedlings were dependent variables and analyzed separately. The effect of the treatments on cotyledonous- and leafy-seedlings mortality were analyzed by a generalized linear model (GLM) with a binomial family test using the R program ver. 3.4.1. Species and treatments were used as independent variables. The dependent variables were the numbers of dead and surviving seedlings. For each species the percent mortality per day of cotyledonous-seedlings and leafy-seedlings in the control treatment (control and open cage) were compared by t-test (two-tailed at significant level of 0.05).

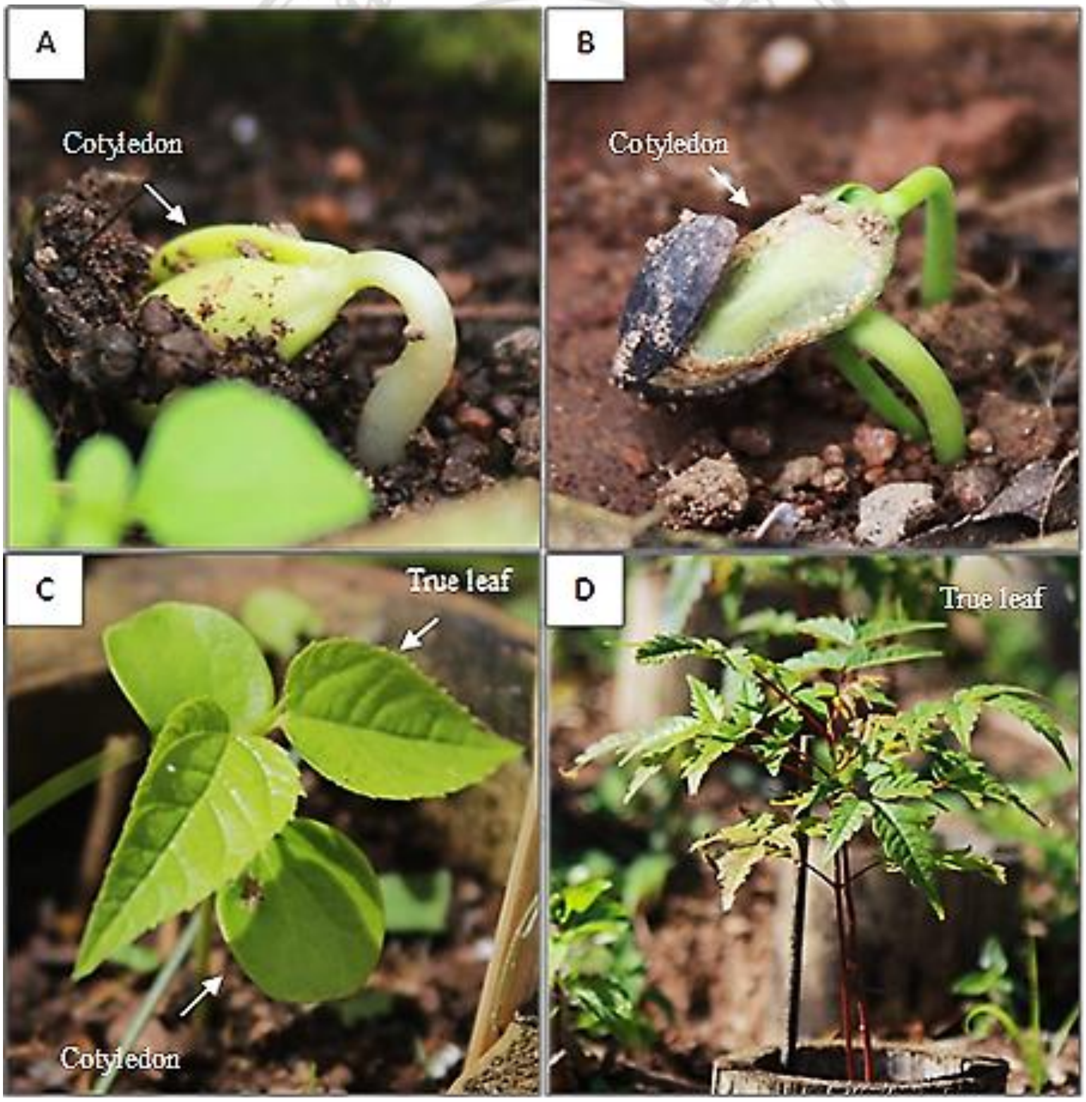


Figure 3.11 Young seedlings of *P. cerasoides* (A) and *A. kurzii* (B) with cotyledons, but no true leaf. Small seedlings with both cotyledons and true leaves of *H. dulcis* (C) and *C. axillaris* (D)

Dead seedlings were classified into three categories according to their appearance:-

1) seedlings with only stem (no leaves present)

2) dry seedlings and

3) nothing observed (no seedling or stem in the bamboo tube).

3.4.3 Seedling survival and relative growth rates

Seedling growth parameters were monitored three times, in October 2015, April 2016 and after the dry season in July 2016 (Table 3.3). Measurements included seedling height, crown width, root collar diameter and health.

For seedling survival, the numbers of seedlings that survived after the dry season in July 2016 were recorded and percent survival of those that had germinated was calculated. A generalized linear model (GLM) with a binomial family test was carried out using the R program ver. 3.4.1 to compare differences among species. Tree species was used as the independent variable. The dependent variable was of mortality.

For seedling growth, the relative growth rate (RGR) of each species was calculated from changes in height (H), root collar diameter (RCD) and crown width (CW) of each surviving tree. The formula followed that of FORRU (2006): -

$$\mathbf{RGR} = \frac{\mathbf{ln\ (final) - ln\ (initial) \times 36{,}500}}{\mathbf{No.\ days\ between\ measurements}}$$

3.4.4 Seedling performance index (SI)

A species performance index (SI) was calculated using plant species traits from the field experiment, a year after sowing seeds (Table 3.2).

Table 3.2 Species performance index (SI)* by seed removal and seedling traits

Features	Categories	Score	Rating score
Seed removal	less than 10%	4	Excellent
	10-35%	3	Acceptable
	34.9-50%	2	Marginal
	more than 50%	1	Unacceptable
MLD**	less than 30 days	4	Excellent
	30-50 days	3	Acceptable
	51-70 day	2	Marginal
	more than 71 days	1	Unacceptable
Germination	more than 70%	4	Excellent
	50-70%	3	Acceptable
	40-49.5%	2	Marginal
	less than 40%	1	Unacceptable
Seedling mortality (% from total germination)	less than 15%	4	Excellent
	14.9-30%	3	Acceptable
	29.9-50%	2	Marginal
	more than 50%	1	Unacceptable
Survival	more than 70%	4	Excellent
	50-69.9%	3	Acceptable
	40-49.9%	2	Marginal
	Less than 40%	1	Unacceptable
Seedling height	more than 50 cm	4	Excellent
	35.0-50.0 cm	3	Acceptable
	20.0-34.9 cm	2	Marginal
	less than 20 cm	1	Unacceptable
Crown width	more than 50 cm	4	Excellent
	35.0-50 cm	3	Acceptable
	20.0-34.9 cm	2	Marginal
	less than 20 cm	1	Unacceptable
RCD	more than 4.50 mm	4	Excellent
	3.25-4.49 mm	3	Acceptable
	2.00-3.24 mm	2	Marginal
	less than 2.00 mm	1	Unacceptable
RGR (% per year)***	more than 100%	4	Excellent
	75 -99 %	3	Acceptable
	50 - 74%	2	Marginal
	less than 50%	1	Unacceptable
Species rating (from total score above)	30 or more than		Excellent
	21 to 29		Good
	12 to 20		Marginal
	11 or less than		Poor

*The index modified from Elliott *et al*., 2003; Lu *et al*., 2016 and Lu *et al*., 2017)

**MLD: median length of dormancy time from sowing to final germination.

***RGR averaged from 3 parts: height, crown width and RCD

3.4.4 Potential seed predators

The photographs from the camera traps were used to calculated an index of species abundance, richness and distribution, based on the assumption that when the population density of animals increases, the possibility of capture by camera traps also increases (Abi-Said and Amr, 2011; Rovero and Marshall, 2009). We used the number of independent photographs to calculate the number per total effort of 100 trap days.

The numbers of individuals, Orders and Families of invertebrate species from pit-fall traps, sticky traps and direct handing were counted. In addition, insects were grouped according to their mouth parts characteristics 1) chewing, 2) sucking and 3) lapping, to indicate their diets.

The abundance of invertebrates (species diversity and evenness) was calculated and determined by Shannon's method (log base e) for each collection period (Shannon and Weaver, 1949). In addition, Sørensen similarity index was used to determine the similarity coefficient of invertebrate communities among three seasons (Diserud and Odegaard, 2007).

5.1 3.4.5 Variation of animal visits and seed-seedling transitional stage

Seed and seedling stage were recorded from the observation. The data were used to create a timeline of seed-to-seedling transition stages. Therefore, relevance between seed-seedling and predator were considered by timeline graph.

CHAPTER 4

Results

4.1 Seed removal

Seed removal of five species was recorded every week. The generalized linear model (GLM) indicated significant effects of species and treatments on seed removal. The five species were divided to two groups: 1) low proportion of seed removal (*H. dulcis*, *A. kurzii*, *P. cerasoides* and *C. axillaris*) and 2) high proportion of seed removal (*H. glabra*) (Figure 4.1). For the former species, the average seed removal in the field of *P. cerasoides*, *H. dulcis*, *A. kurzii* and *C. axillaris* were 0.67 ± 0.67, 1.11 ± 0.86, 1.11 ± 0.61 and 2.44 ± 0.96 percent, respectively (Figure 4.1, C). In the GLM, differences in the proportion of seed removal among the four species were not significant. The predicted probability of seed removal of the species in the control treatment varied from 0.005 to 0.018 (Figure 4.2, Appendix C).

H. glabra was the only species with high seed removal. The average seed removal in the field was 85.78 ± 11.41 percent (Figure 4.1, A). The GLM showed that the probability of *H. glabra* seeds being removed in the control treatment was about 206 times greater than that of *P. cerasoides, H. dulcis, A. kurzii,* and *C. axillaris* (Coefficient estimate ± SE = 10.563 ± 1.785, z = 5.918, P < 0.001).

Comparisons among treatments showed that the cage treatment significantly decreased the proportion of seed removal compared with the control (Coefficient estimate ± SE = -5.583 ± 1.618, z = -3.450, P < 0.001). The insecticide plus cage treatment was marginally effective at protecting seeds from being removed. The open cage, and insecticide treatment did not prevent seed removal significantly (Figure 1).

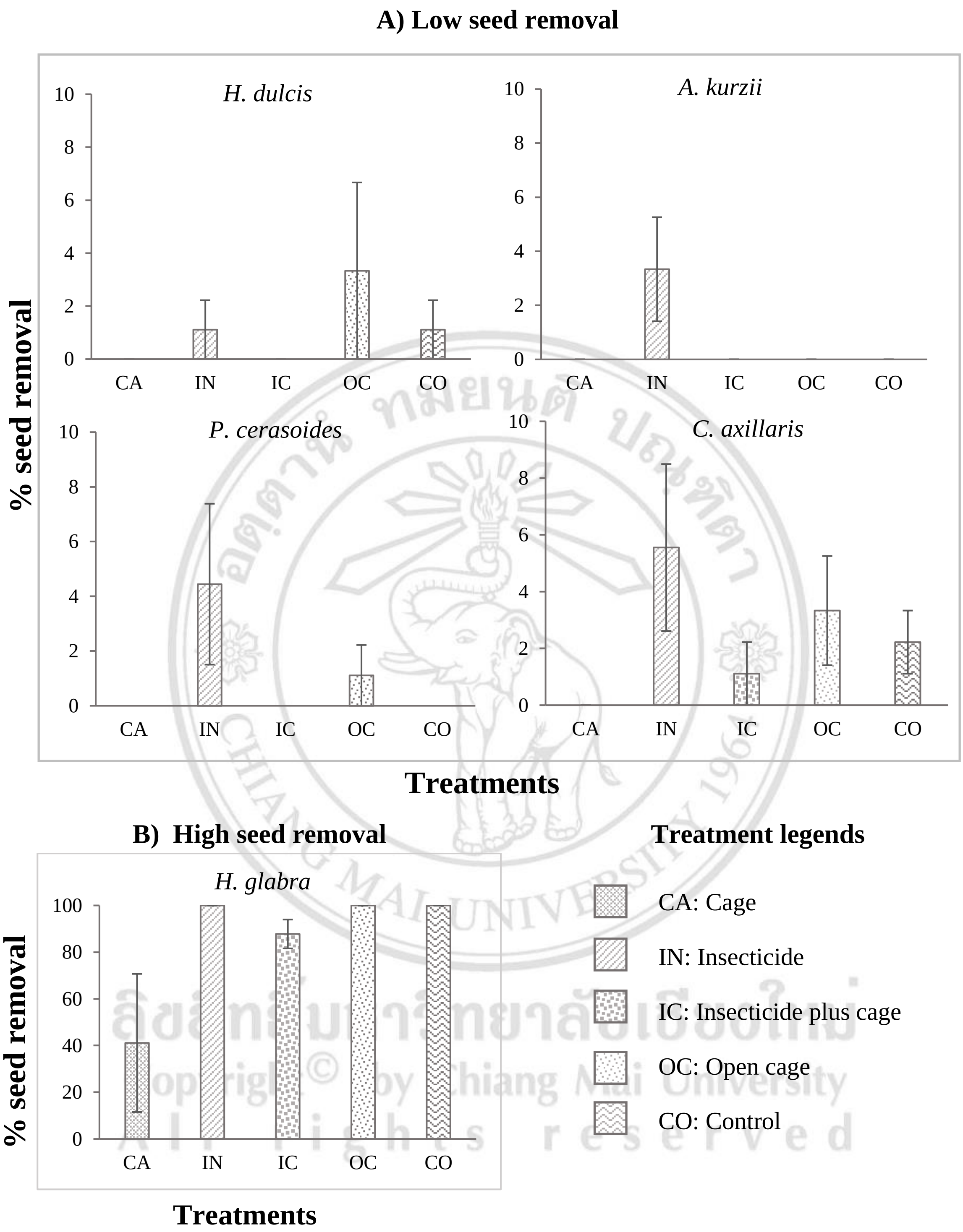


Figure 4.1 Actual percent seed removal from the field data (three replicates of 30 seeds per replicate). Tree species were categorized into two groups according to the generalized linear model (GLM). Each graph shows percent seed removal (±1SE) in five treatments; CA: cage ( ), IN: insecticide ( ), IC: insecticide plus cage ( ), OC: open cage ( ), CO: control ( ).

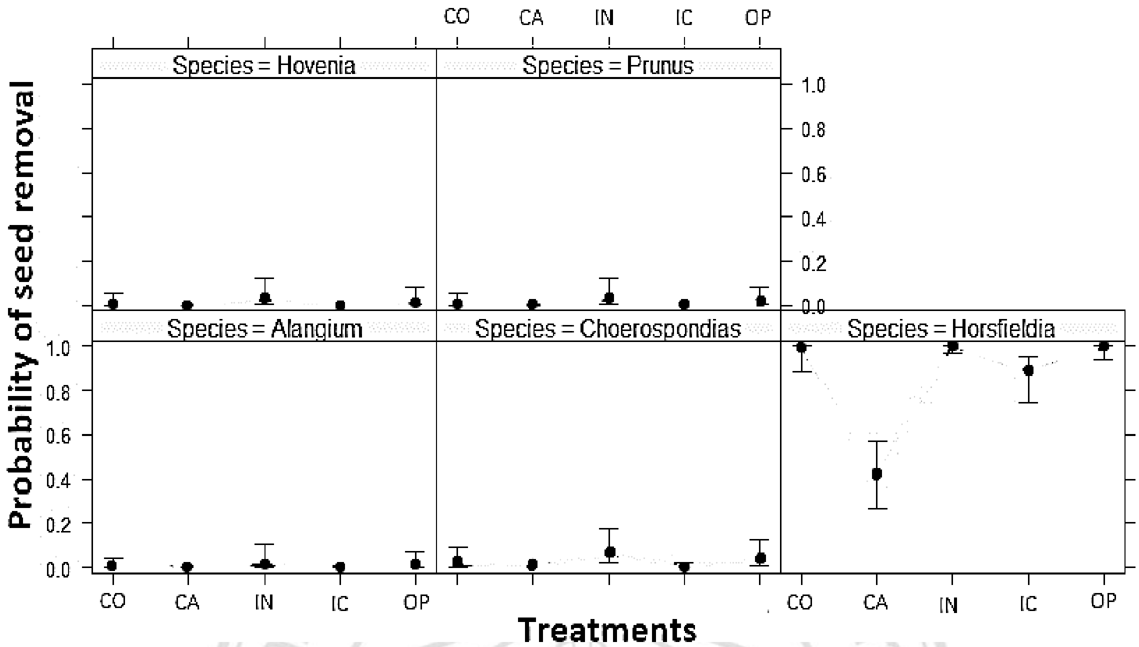


Figure 4.2 Effect plots represent the proportion of seed removal predicted from the generalized linear model (GLM). Each panel shows the prediction (±1SE) of each tree species in five treatments including CO (control), CA (cage), IN (insecticide), IC (insecticide plus cage) and OP (open cage).

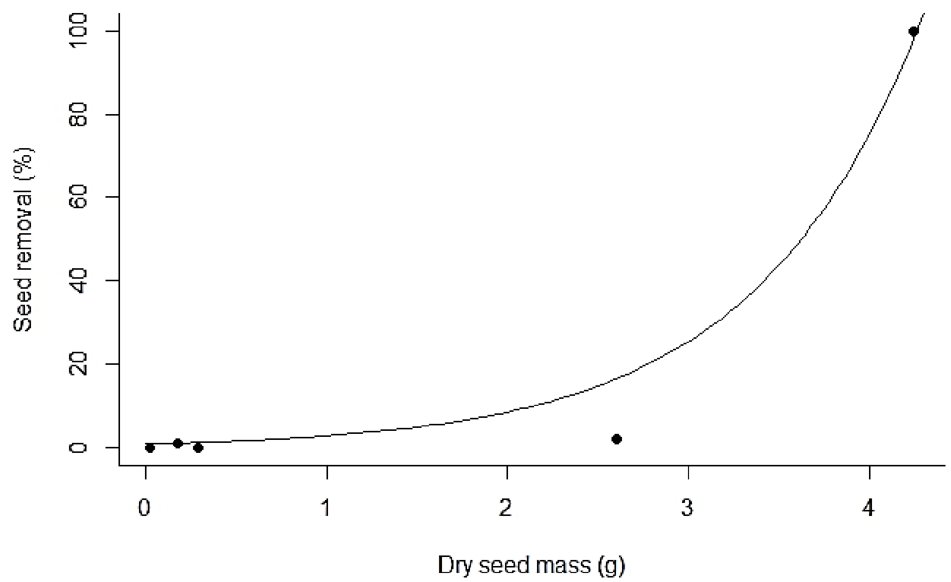


Figure 4.3 Relationship between the percent seed removal and dry seed mass (the non-linear equation: seed removal (y) = e $^{(1.081x)}$, residual standard error = 7.302 on 4 df and p-value < 0.001)

In addition, the relationship between seed mass and mean percent seed removal (control treatment) was determined by a non-linear regression through the origin. The mean percent removal was increased with increase in seed mass (coefficient estimate ± SE = 1.081 ± 0.017, t = 62.25, P = 0.0271; Figure 4.3)

4.2 Seed germination

Seed germination in the field

In the field experiment, percent seed germination was calculated as the number of germinated seeds, divided by the number of seeds that remained after seed removal. For *H. glabra* no seeds germinated in the field. Therefore, *H. glabra* was not included in the analysis of seed germination and *H. glabra* was classified into no germination group (Figure 4.4 - 4.5).

For the other four species, the GLM showed that treatments had no effect on the proportion of seeds that germinated in comparison with the controls (see in Appendix C). Averaging across species, seed germination was 44.27 ± 8.40 percent with insecticide, 45.80 ± 11.19 percent for the control, 47.62 ± 17.54 percent in open cages, 53.74 ± 14.84 percent with insecticide plus cages and 54.72 ± 19.04 percent in closed cages.

The GLM indicated germination ability differed significantly among the four tree species. *A. kurzii* (73.55 ± 5.61 percent) and *P. cerasoides* (72.54 ± 5.19 percent) germinated the most (Coefficient estimate ± SE = 1.012 ± 0.212, z = 4.762, *P*-value < 0.001). *C. axillaris* (33.36 ± 2.80 percent) germinated morderately, whilest *H. dulcis* germinated the least with 17.46 ± 2.12 percent germination (Figure 4.4). The predicted probability of seed germination of tree species in the control treatment varied from 0.17 to 0.73 (Figure 4.5, Appendix C).

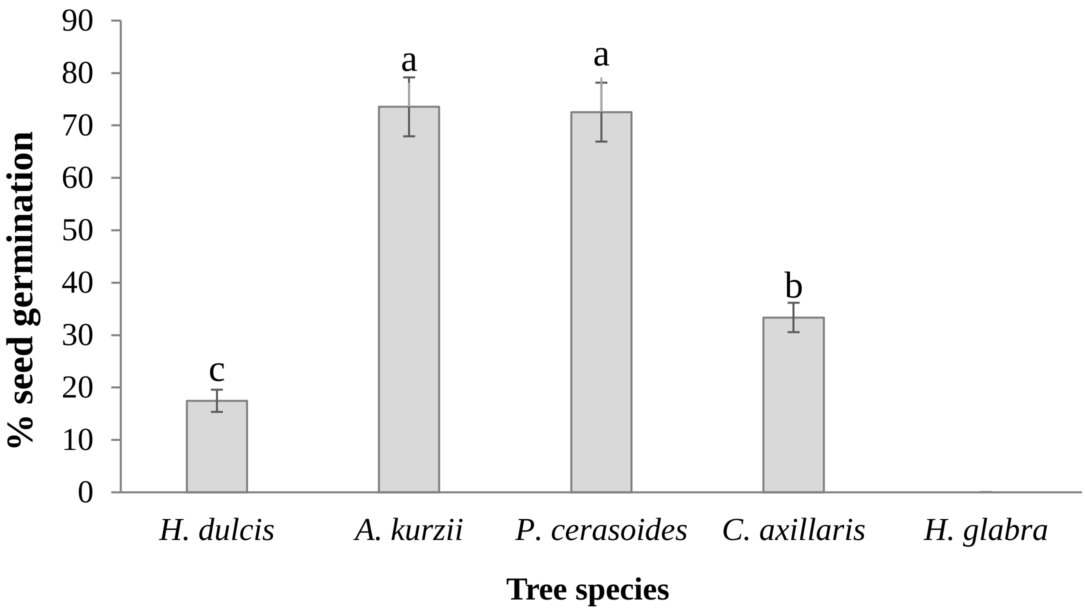


Figure 4.4 Actual percent seed germination (±1SE) from the field data (three replicates of 30 seeds per replicate) averaged across all treatments of the five studied species. The letter (a - c) represent significant differences among tree species compared with *H. dulcis* according to the generalized linear model (GLM).

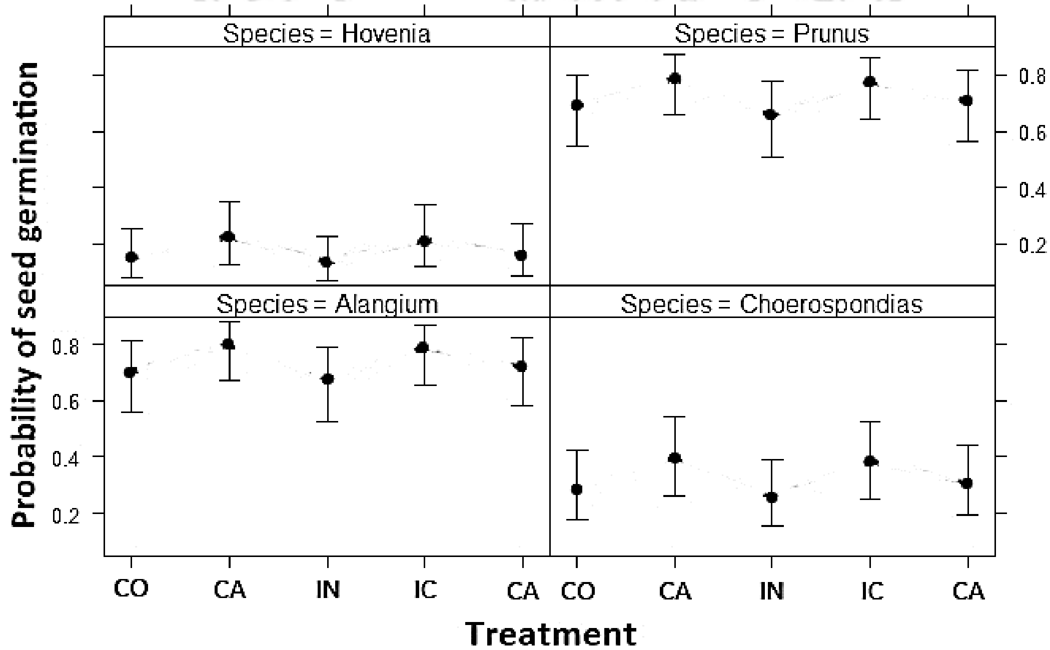


Figure 4.5 Effect plot represents the proportion of seed germination predicted from the GLM. Each panel shows the prediction (±1SE) of each tree species in five treatments, including CO (control), CA (cage), IN (insecticide), IC (insecticide plus cage) and OP (open cage).

Seed germination in the nursery

The insecticide treatment had no significant effect on seed germination. Survival analysis (N = 90 seeds per treatment) showed no significant difference between the insecticide spraying and the control treatment (Chi-square < 3.84, at significant level of 0.05) for the four species (Figure 4.6).

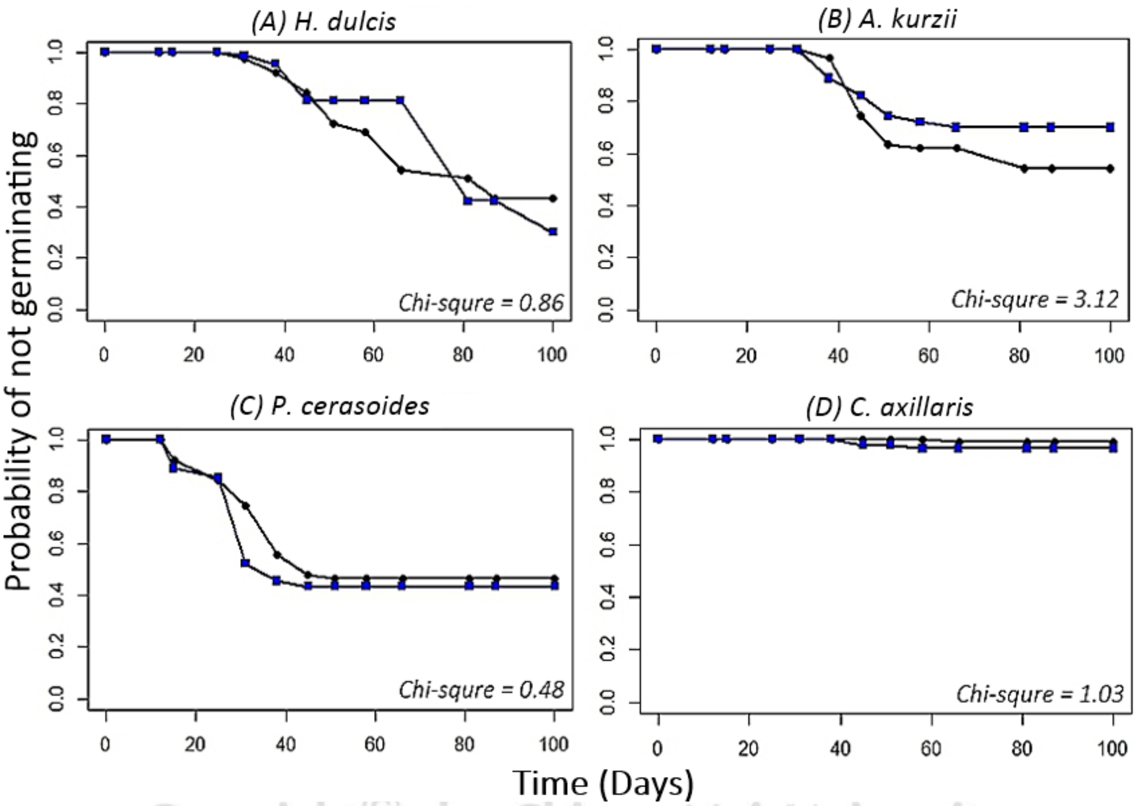


Figure 4.6 Survival plot showed probability of not germinating of seeds in two treatments: insecticide spraying (—■—) and no insecticide apply (control; —●—). Log-rank test (Chi-square test, *df* = 1, Critical value = 3.84) was used to determine the difference between treatments.

Germination between nursery and field

For each species the percent seed germination in the control treatment of the field experiment was compared to that of the nursery experiment. *H. dulcis* had significantly higher germination in the nursery (60.00 ± 4.84 percent) than in field (17.78 ± 2.22 percent). Unlike *H. dulcis,* C. *axillaris* and *A. kurzii* had higher germination in field than in the nursery experiment (Chi-square < 3.84, at significant level of 0.05). Seed germination of *P. cerasoides* was more than 70 percent in both field and nursery experiments and did not differ between the two conditions (Chi-square = 2.92 at significant level of 0.05) (Figure 4.7).

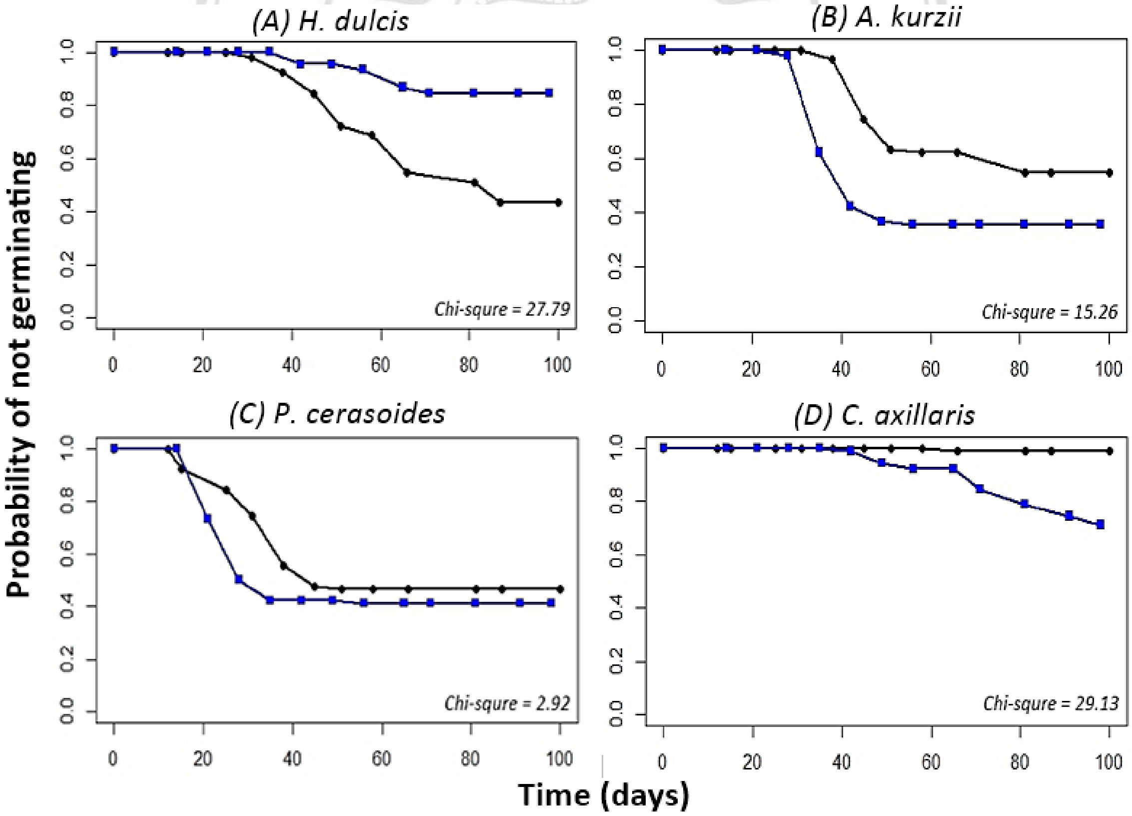


Figure 4.7 Survival plot showed probability of not germinating of seeds in two conditions: field experiment (—■—) and in tree-nursery (control; —●—). Log-rank test (Chi-square test, *df* = 1, Critical value = 3.84) was used to determine the difference between experiments.

4.3 Cotyledonous-seedling and leafy-seedling mortality

Cotyledonous-seedling mortality

The GLM indicated that the species and treatments affected seedling mortality. However, the effect of species was statistically marginal. Among species, the mean percent mortality of cotyledonous-seedlings ranged from 0.71 ± 0.71 percent (*H. dulcis*) to 7.92 ± 2.35 percent (*P. cerasoides*). The cotyledonous-seedling mortality of *C. axillaris*, and *H. dulcis* was marginally lower than that of *A. kurzii*, and *P. cerasoides* (Figure 4.8; Appendix C).

Among treatments, the GLM showed that the cage and the insecticide plus cage treatments significantly decreased mortality compared with the control (Coefficient estimate of cage treatment ± SE = -1.729 ± 0.656, z = -2.634, *P*-value = 0.008; coefficient estimate of insecticide plus cage treatment ± SE = -1.3592 ± 0.5897, z = -2.305, *P*-value = 0.02). The probability of cotyledonous-seedlings dying in the cage treatment was 0.18 times lower than that in the control with the insecticide plus cage treatment, the probability of dying was 0.26 times lower than that in the control treatment (Figure 4.9).

Leafy Seedling mortality

Leafy-seedling mortality ranged from 11.93 ± 2.53 percent for *C. axillaris* to 40.80 ± 9.80 percent for *H. dulcis* (Figure 4.10). The GLM indicated that the effect of the treatments on the leafy-seedling mortality was insinificant (Appendix C), but leafy-seedling mortality did differ among species. *H. dulcis* had the highest leafy seedlings mortality (Coefficient estimate ± SE = -2.032 ± 0.790, z = -2.572, P-value = 0.010). Prediction model from GLM showed probability of leafy seedling mortality in *H. dulcis* was 0.024 across treatments (Figure 4.11, Appendix C).

Comparison of cotyledonous-seedling and leafy-seedling mortality

Across species, the average percent mortality per day was higher in the cotyledonous-seedling stage (0.59 ± 0.21 percent per day) than the leafy-seedling stage (0.15 ± 0.05 percent per day) (Figure 4.12). For *H. dulcis* and *C. axillaris,* the seedling mortality was not significantly different between the two stages (see in Appendix C). On the other hand, *P. cerasoides* and *A. kurzii* had significantly higher percent cotyledonous-seedling mortality per day compared with the percent leafy-seedling mortality (t = 2.674, df = 5.176, p-value = 0.043 for *A. kurzii* and t = 2.978, df = 5.015, p-value = 0.031 for *P. cerasoides*) (Figure 4.12).

Cause of seedling mortality

In addition, physical appearance of dead seedlings was examined to infer causes of dead in the field. There were three categories of seedlings - 1) seedlings with only stem and leaves absent), 2) dried-out seedlings, and 3) disappeared seedlings (no remaining stem in the bamboo tube) (Figure 4.13). Approximately, Two percent of all dead seedlings showed the signs of leaf removal, while six percent of all dead seedlings had desiccated. The majority of seedlings assumed dead were disappeared from the bamboo tube.

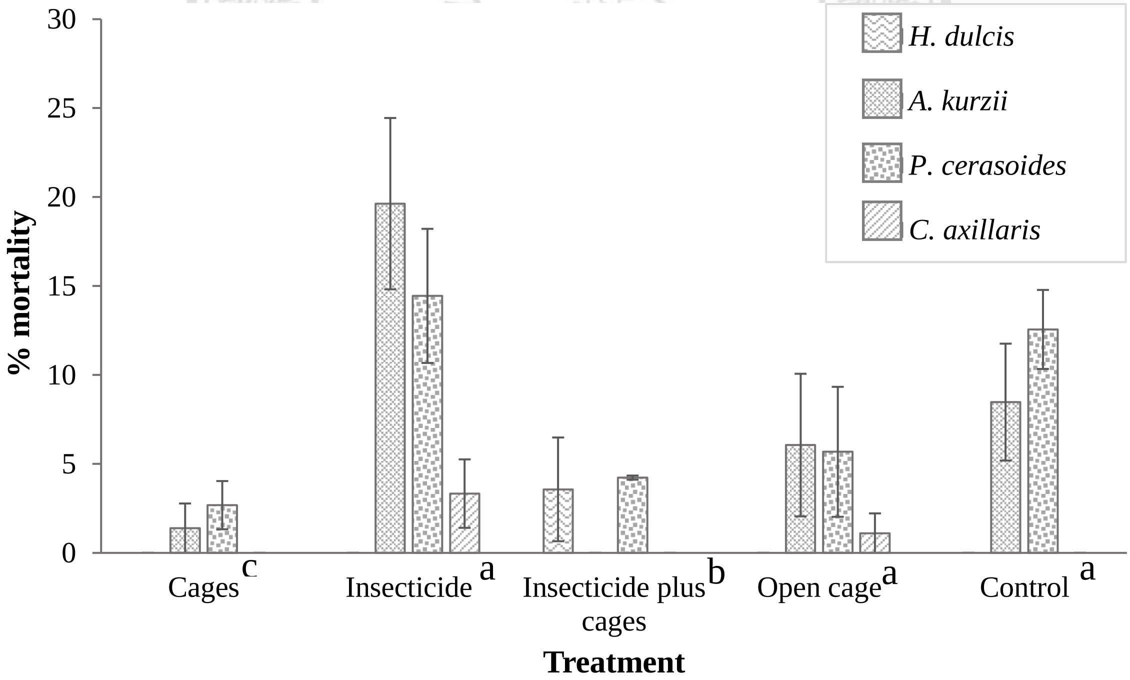


Figure 4.8 Actual percent cotyledonous-seedling mortality (±1SE) from the field data (three replicates of 30 seeds per replicate) of four tree species (*H. dulcis* ( ), *A. kurzii* ( ), *P. cerasoides* ( ) and *C. axillaris* ( )) calculated from total germination. Five treatments were categorized into groups according to the GLM. The letters (a - c) indicate significantly different proportions of cotyledonous-seedling mortality, compared with control treatment.

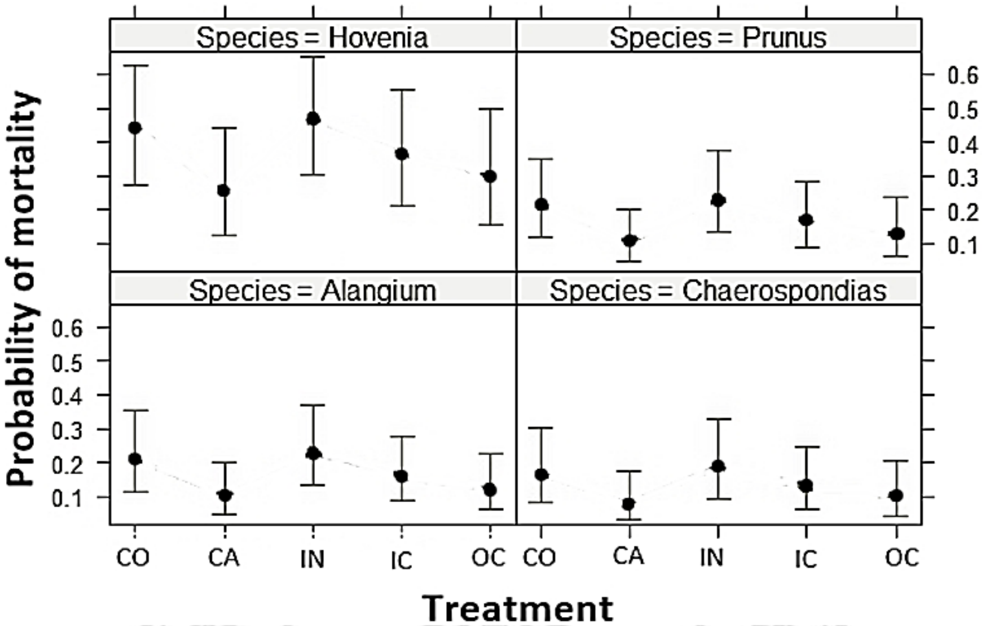


Figure 4.9 Effect plot represent the proportion of cotyledonous-seedling mortality predicted by GLM. Each panel shows a prediction of mortality probability (±1SE) for each tree species in five treatments, including CO (control), CA (cage), IN (insecticide), IC (insecticide plus cage) and OP (open cage).

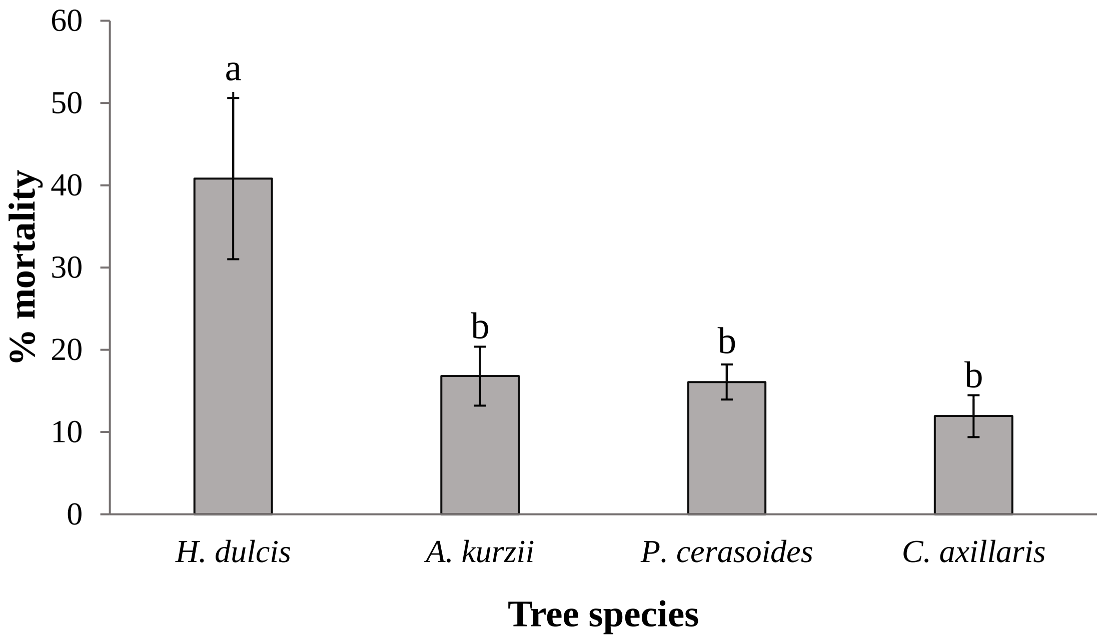


Figure 4.10 Actual mean percent leafy-seedling mortality (±1SE) of four tree species average over five treatments (30 seeds per replicate 0f three), calculated from total germination. The letters (a – b) indicated significantly different proportions of mortality, compared with *A. kurzii* species, estimated by the GLM (at p-value = 0.05).

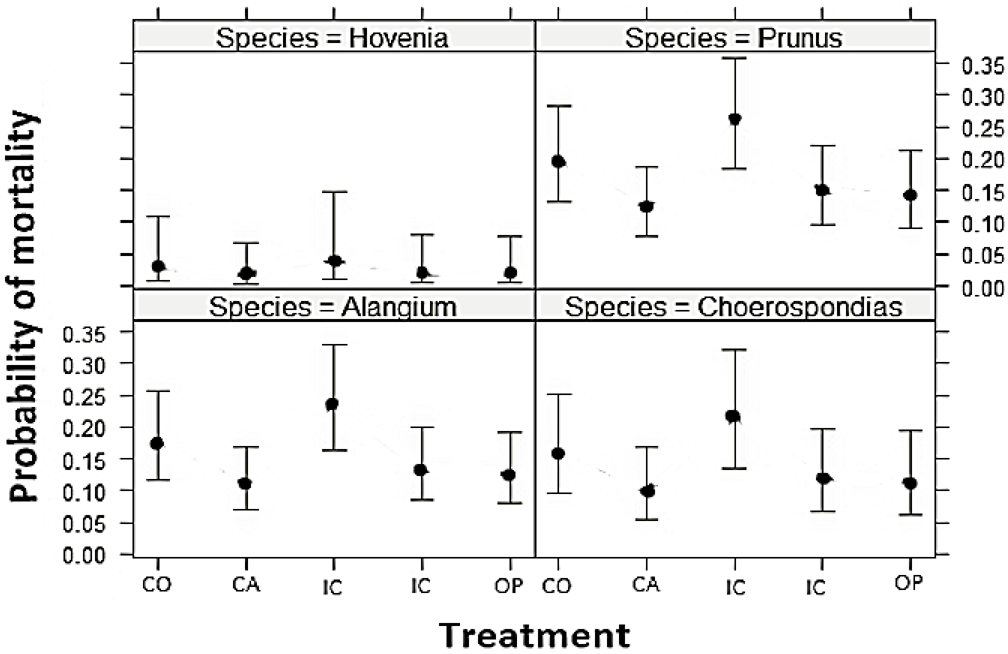


Figure 4.11 Effect plot represent the proportion of leafy-seedling mortality predicted by GLM. Each panel shows prediction (±1SE) of each tree species in five treatments including CO (control), CA (cage), IN (insecticide), IC (insecticide and cage) and OP (open cage).

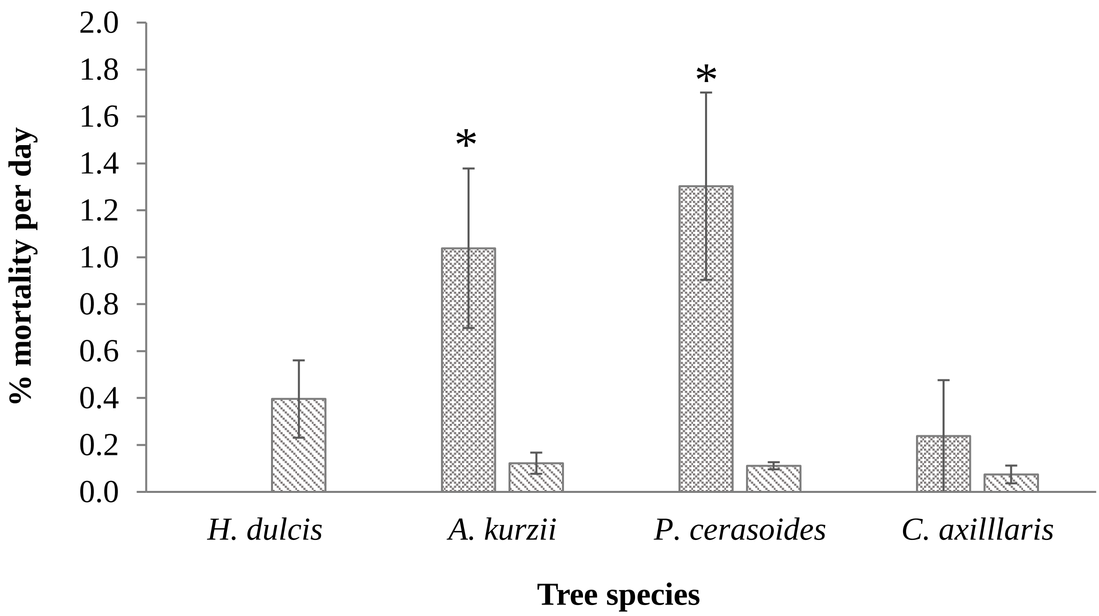


Figure 4.12 Comparing of percent mortality per day between cotyledonous-seedling ( ) and leafy-seedling stages ( ) of each species by t-test (at p-value less than 0.05), * represent significant higher percent mortality per day (total day: 7 days for cotyledonous-seedling and 139 days for leafy-seedling).

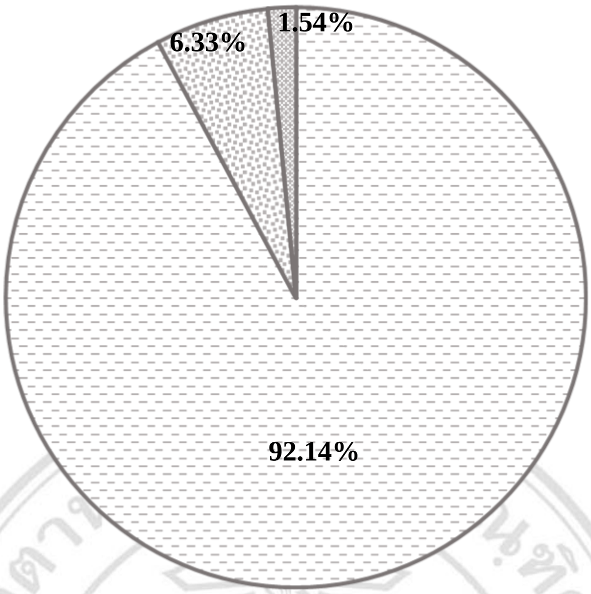


Figure 4.13 Three categories of physical appearance and percent of seedlings found. Percent cause of seedling mortality with only stem ( ), dry seedling ( ) and nothing can observed ( )**.**

4.4 Seedling survival

After the predator exclusion experiments had been terminated, seedling survival continued to be monitored until July 2016 (after dry season). The mean percent seedling survival ranged from 13.49 ± 8.29 percent (*H. dulcis*) to 56.74 ± 6.04 percent (*P. cerasoides*). The GLM showed that *P. cerasoides* and *C. axillaris* survived significant better survive than *A. kurzii* and *H. dulcis* did (Figure 4.14, appendix C). The mean predicted probability of survival of *P. cerasoides* and *C. axillaris* seedling was 0.58, while it was 0.22 in *A. kurzii* and *H. dulcis* (Figure 4.15).

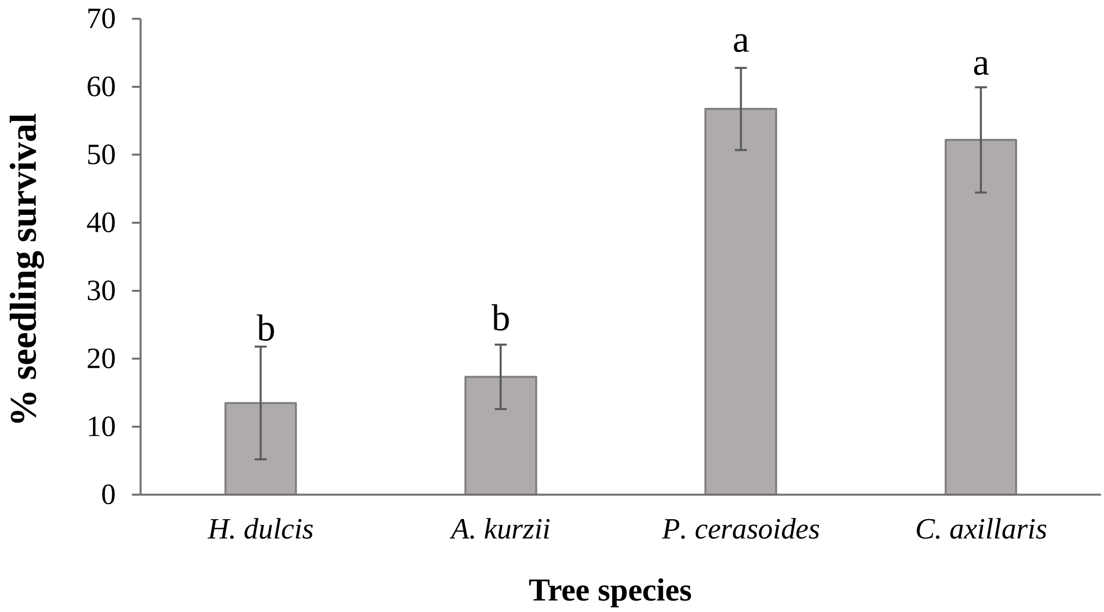


Figure 4.14 Observed mean percent seedling survival of four tree species; *H. dulcis A. kurzii*, *P. cerasoides*, *C. axillaris* and (10-month old seedlings). The letters (a - b) indicate differences in proportion of seedling surviving at p-value = 0.010 according to the GLM.

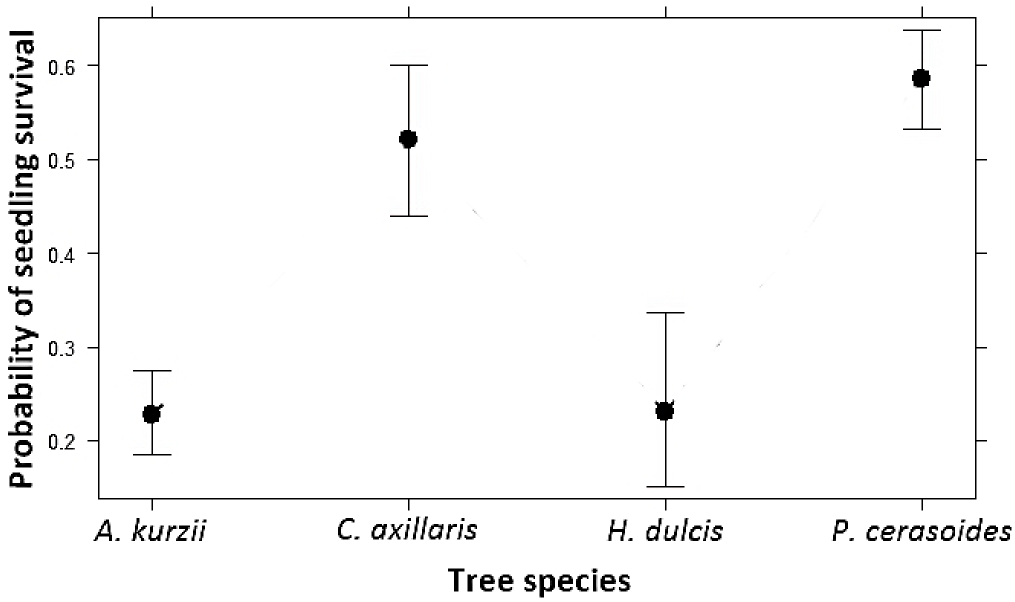


Figure 4.15 Effect plot represents the proportion of seedling survival (±1SE) predicted by the generalized linear model (GLM).

4.5 Growth and performance index

Among the species tested *P. cerasoides* seedlings grew the tallest, had the broadest crown (CW), and the largest root collar diameter (RCD) by the end of the study period. The relative growth rates (RGR) of each growth measurement varied among species. Using relative height growth, the fastest growing species were *P. cerasoides* and *C. axillaris*; the two species grew more than 450% per year (Table 4.1). For the relative CW growth, *C. axillaris* had the highest RGR. In contrast, despite its small size, *H. dulcis* had the highest relative RCD growth among the four species.

Relative performance score for the five tree species were calculated by combining nine parameters (see in Table 3.2). Only one species, *P. cerasoides*, was classified as an excellent species. The pioneer tree species, *P. cerasoides* had low seed removal, high seed germination and high survival and growth. *A. kurzii* and *C. axillaris* had good performance for direct seeding. *H. dulcis* was classified as a marginal species because of low germination and small seedling size. The species with the poorest performance *H. glabra* is not recommended for direct seeding.

Table 4.1 Mean growth measurements - height, crown width (CW) and root collar diameter (RCD) - and relative growth rate (RGR: percent per year) of the growth measurements. The growth of *H. glabra* was not available due to no seed germination and seedling establishment.

Species name	N	Height		CW		RCD	
		Mean ± SE (cm)	%RGR (per year)	Mean ± SE (cm)	%RGR (per year)	Mean ± SE (mm)	%RGR (per year)
H. dulcis	15	15.57 ± 0.80	281.86	13.91 ± 1.61	287.77	2.17 ± 0.13	367.50
A. kurzii	73	16.41 ± 1.05	238.31	14.72 ± 1.14	184.48	2.93 ± 0.14	227.52
P. cerasoides	184	66.51 ± 5.19	457.22	29.84 ± 1.20	257.91	4.69 ± 0.23	222.40
C. axillaris	45	32.23 ± 3.00	458.68	23.41 ± 0.97	432.85	2.72 ± 0.15	181.10
H. glabra	-	-	-	-	-	-	-

Table 4.2 Summary of tree species performance score and classification based on direct seeding field (rating score: E (excellent), A (acceptable), M (marginal) and U (unacceptable)). (See Table 3.2 for definitions)

Parameters	*H. dulcis*		*A. kurzii*		*P. cerasoides*		*C. axillaris*		*H. glabra*	
	Score	*Rating score*	*Score*	*Rating score*	*Score*	*Rating score*	*Score*	*Rating score*	*Score*	*Rating score*
%Seed removal	4	E	4	E	4	E	4	E	1	U
%Seed germination	1	U	4	E	4	E	1	U	1	U
MLD	4	E	4	E	4	E	4	E	1	U
%Seedling mortality	2	M	3	A	3	A	4	E	-	-
%Survival (after dry season)	1	U	1	U	3	A	3	A	-	-
Height	1	U	1	U	4	E	2	M	-	-
CW	1	U	1	U	2	M	2	M	-	-
RCD	2	M	2	M	4	E	2	M	-	-
Average of RGR (%per year)	4	E	4	E	4	E	4	E	-	-
Species performance	20	**Marginal**	24	**Good**	32	**Excellent**	26	**Good**	3	**Poor**

4.6 Potential seed predators

a. Vertebrates species (Bird and small mammals)

Over the course of the seven months, each camera was installed for 200 trap days, for a total of 1,000 trap days over all. Fifteen animal species were detected in 116 photographs. The total number of animal photographs was highest in the first month. Among all the photographs, 54% were of two seed predator species: rat (*Rattus sp.*) and barred buttonquail (*Turnix suscitator*). Thirteen species of non—seed predators were also photographed, accounting for 46% of the total number of photographs.

Most animals visited the plots during the daytime whilst only 3 species, rat (*Rattus sp*.), hog badger (*Arctonyx collaris*), and the large Indian civet (*Viverra zibetha*) visited at night. Among the detected animals, rodents were detected more frequently than other small mammals and bird species (Table 4.3).

Table 4.3 Relative species occurrence in each month shown by the number of photographs per total effort 100 trap days (R) and the percentage of total (%). The number in parenthesis under the month name shows total trap days in each month of five cameras.

Scientific name	Common name	Aug (140)		Sep (175)		Oct (140)		Nov (140)		Dec (175)		Jan (140)		Feb (90)	
		Ni	R	Ni	R	Ni	R	Ni	R	Ni	R	Ni	R	Ni	R
Seed predator															
Rattus sp.	Rat	30	273	2	6	3	19	6	46	0	0	0	0	7	61
Turnix suscitator	Barred Buttonquail	18	72	1	1	0	0	0	0	0	0	0	0	0	0
Sum Predator		**48**		**3**		**3**		**6**		**0**		**0**		**7**	
% in month		**67.606**		**30**		**33.333**		**46.154**		**0**		**0**		**41.176**	
% in group (all month)		**71.642**		**4.4776**		**4.4776**		**8.9552**		**0**		**0**		**10.448**	
Non_Seed predator															
Anthus cervinus	Red-throated Pipit	0	0	0	0	1	4	0	0	0	0	0	0	0	0
Tupaia belangeri	Northern tree shrew	6	14	1	3	1	11	4	16	0	0	2	29	0	0
Canis aureus	Asia jackal	0	0	0	0	1	14	0	0	0	0	0	0	0	0
Centropus sinensis	Greater coucal	6	119	2	6	1	17	0	0	0	0	1	4	2	17
Herpestes javanicus	Small Asian Mongoose	2	7	1	3	1	11	1	4	0	0	0	0	3	8
Lanius schach	Long-tailed Shrike	0	0	0	0	1	4	1	4	0	0	0	0	1	4
Lonchura punctulata	Scaly-breasted Munia	0	0	0	0	0	0	0	0	0	0	0	0	1	11
Prionailurus bengalensis	Leopard cat	2	4	3	9	0	0	0	0	0	0	0	0	1	11
Phylloscopus trochiloides	Greenish Warbler	0	0	0	0	0	0	0	0	0	0	0	0	1	4
Pycnonotus aurigaster	Sooty-headed bulbul	0	0	0	0	0	0	1	4	0	0	3	18	0	0
Saxicola caprata	Pied Bushchat	3	3	0	0	0	0	0	0	0	0	1	4	1	6
Arctonyx collaris	Hog badger	3	6	0	0	0	0	0	0	0	0	0	0	0	0
Viverra zibetha	Large Indian civet	1	7	0	0	0	0	0	0	0	0	0	0	0	0
Sum Non_Seed predator		**23**		**7**		**6**		**7**		**0**		**7**		**10**	
% in month		**32.394**		**70**		**66.667**		**53.846**		**0**		**100**		**58.824**	
% in group (all month)		**38.333**		**11.667**		**10**		**11.667**		**0**		**11.667**		**16.667**	
	All	**71**		**10**		**9**		**13**		**0**		**7**		**17**	

b. Invertebrate species (Insects)

Collected invertebrates (insect) were classified into Order and Family. The total number of insect was 6,170 individuals from 73 families and 17 orders (Table 4.4). Species in Order Hymenoptera were the most commonly capture individuals out of a total of 3,544 individuals from 11 families. They were dominant in every period. Followed by Order Diptera (1,284 individuals, 13 families), Order Homoptera (398 individuals, 6 families) and Order Coleoptera (162 individuals, 8 families). Whereas, less than 100 number of individuals of other insect orders were caught representing few number of family.

Insects were divided to 3 groups according to their diet feeder (Table 4.4):-

Insect seed predators, included 3 Families from 3 Orders. Ant species (Order Hymenoptera; Family Formicidae) was the most dominant of this insect group. Other species were in family Curculionidae (Order Coleoptera) and Largidae (Order Hemiptera).

Insect plant feeders included 24 Families from 9 Orders. Thrips (Order Thysanoptera; Family Phlaeothripidae) was the most abundant for plant feeder group followed by Leafhoppers (Order Homoptera; Family Cicadellidae) and Aphids (Order Homoptera; Family Cicadellidae).

Other insect groups were predators of other insects, scavengers and parasitoids. This group comprised 42 families from 13 orders. Most of them were in the Orders Diptera and Hymeoptera.

They were classified into three groups according to their mouthparts, 1) chewing, 2) sucking and 3) lapping mouthpart. Sixty-one percent of collected families had chewing mouthparts from 36 families. Twenty and 19% of collected individuals had lapping and sucking mouthpart types, respectively (Figure 4.16).

The species composition of the insects captured varied among collection periods. Species richness, diversity and species evenness were highest all in August 2015, followed by October 2015 and dry season on April 2016 respectively (Table 4.4).

Sorensen's coefficient similarity index ranged from 0 (the lowest similarity between two communities) to 1 (vary high similarity between two communities or both communities are same). The insect community composition in August 2015 was more differ from April 2016 and October 2015 when compared among three months (Table 4.4). However, the coefficient range from 0.514 - 0.635 which is not much difference. This result can assume the insect community composition did not change much over the study period.

Table 4.4 Number of insect individuals on August 2015, October 2015 and April 2016 (Feeder: S (seed feeder/destroyer), P (plant feeder) and O (other insect or predator of other insects))

	Order	**Family**	**Aug 2015**	**Oct 2015**	**Apr 2016**	**Feeder**
1	Araneae	Araneae	6	22	21	O
2		Unknown	2	1	1	O
3	Blattodea	Blaberidae	1			O
4		Blatellidae		4	3	O
5		Unknown		8		O
6	Coleoptera	Carabidae	1	1		P
7		Chrysomelidae	1	2		P
8		Curculionidae	4	1	2	P/S
9		Leidodidae	1			P
10		Scarabaeidae	81			P
11		Schizopteridae			3	P
12		Staphylinidae	7	4		O
13		Unknown	42	10	2	O
14	Collembola	Entomobryidae	2	12	7	O
15	Dermaptera	Forficulidae	1	1		O
16		Unknown		1		O
17	Diptera	Calliphoridae	1			O
18		Cecidomyiidae		1		O
19		Chloropidae		1		P
20		Dolichopolidae	19			O
21		Drosophilidae	252			O

Table 4.4 (continued)

	Order	Family	Aug 2015	Oct 2015	Apr 2016	Feeder
22		Faniidae	1			O
23		Leptocera	12			O
24		Muscidae	8			O
25		Phoridae		1		O
26		Platystomatidae	2			O
27		Sarcophagidae	1			O
28		Tachinidae	2			O
29		Unknown	798	137	48	O
30	Hemiptera	Cimicidae	1			O
31		Largidae		1		P/S
32		Miridae		1	1	P
33		Pentatomidae			1	P
34		Reduviidae	2	3		O
35		Rhopalidae	1			P
36		Schizopteridae			2	O
37		Unknown	3	3	1	O
38	Homoptera	Aphididae	157	10	2	P
39		Cicadellidae	73	70	83	P
40		Cimcidae	1			P
41		Cixiidae (Nymp)	2			P
42	Hymenoptera	Apidae	1			O
43		Bethylidae	1			O
44		Braconidae	3			O
45		Ceraphronidae		2		O
46		Diapriidae		7		O
47		Evaniidae		1		O
48		Formicidae	733	1774	748	S
49		Ichneumonidae	1			O
50		Pompilidae		1		O
51		Tenthredinidae		1		O
52		Unknown	173	37	61	O
53	Isoptera	Termitidae	1	1	1	O

Table 4.4 (continued)

	Order	Family	Aug 2015	Oct 2015	Apr 2016	Feeder
54	Lepidoptera	Erebidae	2	4		P
55		Geometridae	25	13	4	P
56		Noctuidae	1			P
57		Unknown	5		2	O
58	Mantodea	Oligonychinae	1			O
59	Orthoptera	Acrididae	8	3	2	P
60		Gryliidae	3	16	9	P
61		Gryllotalpidae	5			P
62		Tetrigidae	6	4	6	O
63		Unknown	2	2	2	O
64	Phasmatodea	Heteronemiidae	1			P
65	Phasmida	Pseodophasmatidae			1	P
66	Strepsiptera	Corioxenidae		1		O
67	Thysanoptera	Phlaeothripidae	130	24	384	P
Total number of individuals			**2587**	**2186**	**1397**	
Number of species			**41**	**28**	**17**	
Shannon's diversity index			**1.418**	**0.362**	**0.408**	
Species evenness			**0.382**	**0.109**	**0.144**	

*Diversity index based on Shannon's method (Log base e)

Table 4.5 Sorensen's Coefficient similarity matrix (data log (e) transformed) showed the number of correspondences among insect community in tree month (calculated by number of individual in families).

	Aug-15	Oct-15	Apr-16
Aug-15	1		
Oct-15	0.552	1	
Apr-16	0.514	0.635	1

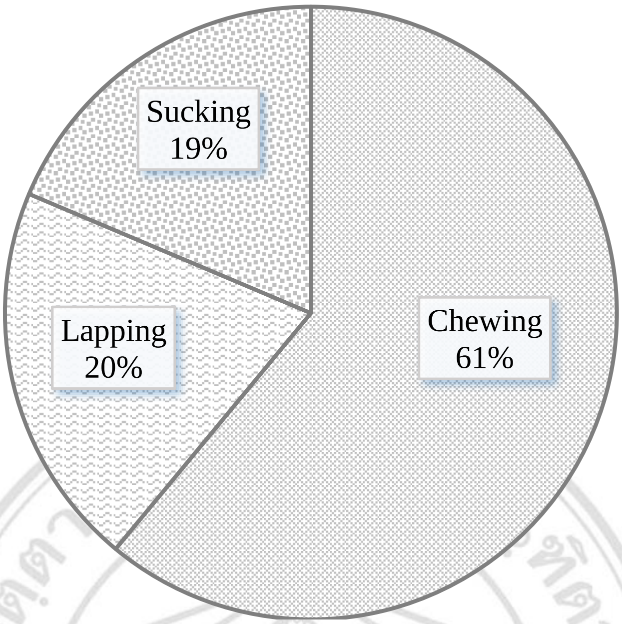


Figure 4.16 Proportion of insect families classified by mouthparts; chewing mouthparts ( ), lapping mouthparts ( ) and sucking mouthparts ( ).

4.7 Variation of animal visits and seed-seedling transitional stage

Vertebrates began visiting the plot two days after seeds were sown in August 2015. Of all seeds, 21.56% were destroyed and/or removed from the bamboo tubes from August through October. No seeds were destroyed and/or removed from November to February. Considering to seedlings, mortality peaked in September to October. The relative occurrence of individuals of seed predator and non—seed predator species visiting the plot varied from month to month. In other words, temporal variation in animal visits were observed in this field (Figure 4.17).

Invertebrates species recorded across three seasons fluctuated. Highest abundance was recorded in August and it declined in April. Insect seed predators were abundant in every season, but abundance was not related with seed-seedling stage. As same as insect herbivore, they were lowest in October, which the most of seedling emerged. So, variation of insect abundance and community composition in related with seed and seedling were still not clear (Figure 4.17).

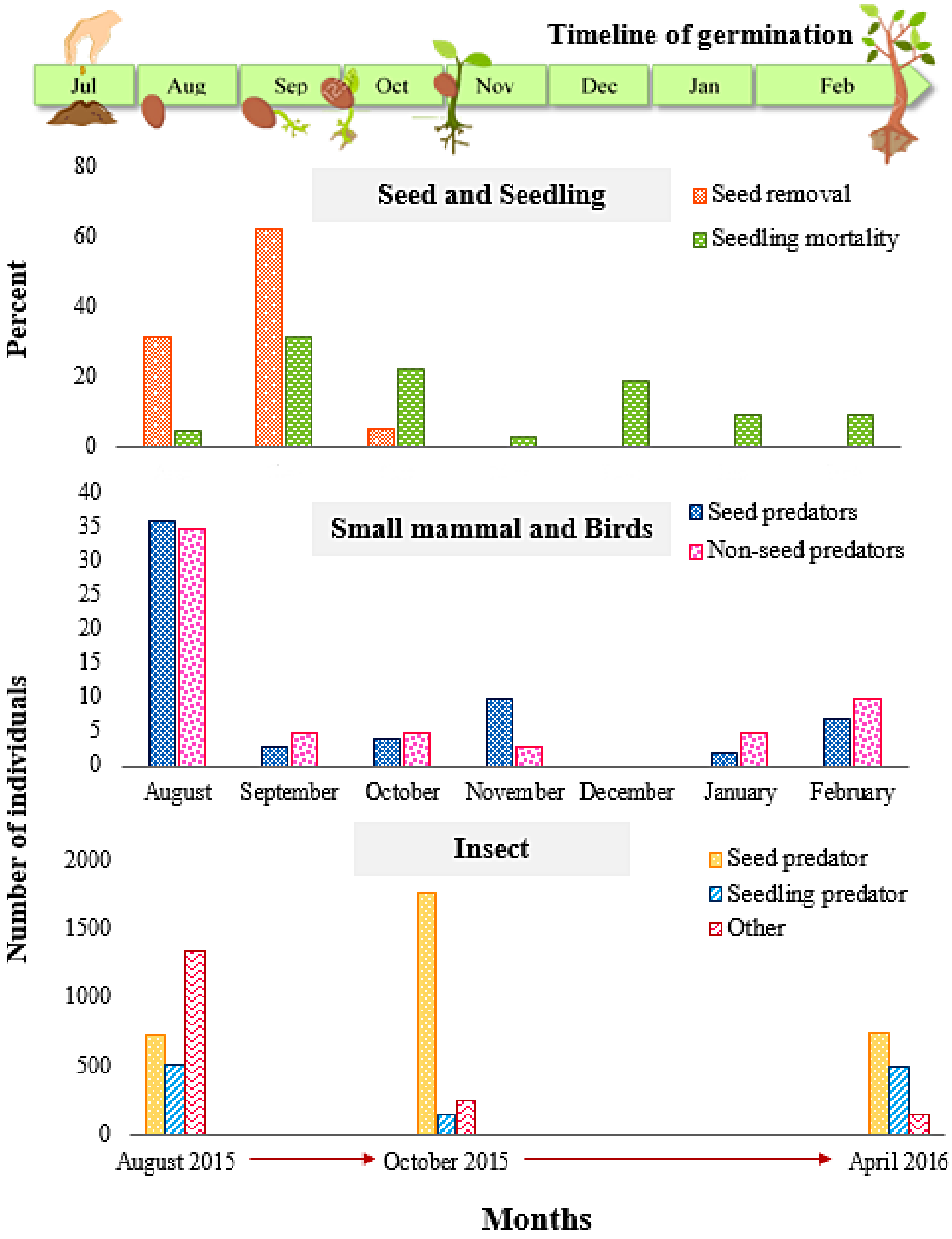


Figure 4.17 The correlation between number of small mammal and birds: seed predators ( ) and non—seed predators ( ) and number of insect: seed predator ( ), seedling predator ( ) and other insect ( ) in related to each seed-to-seedling stage from August 2015 – February 2016.

CHAPTER 5

Discussions

5.2 Seed removal

Variation of seed removal, among tree species was influenced by seed size. In this study, *H. glabra*, the largest seeded species (4.25 g seed dry mass), had the highest percent seed removal (Figure 5.1). Whilst seed removal of the other four studied species (0.024 – 2.602 g seed dry mass), Neotropical savannas was significantly lower. This agrees with a study in by Ferreira *et al*. (2011). They found that seed removal of larger seeded species was much higher than that of smaller seed species (seed mass ranged from 0.025 – 0.400 g) (Ferreira *et al*., 2011). Similarly removal large nuts (weight 7 g to 22 g) is much higher than of smaller seeds (90 percent) at the end of experiment (Brewer, 2001). When both small and large seeds are available, animals selectively consume the large seeded species (Sobral *et al*., 2013). Consumption of large seeds with rich nutrient seed reserves allows animals to gain more energy per unit search time (Brewer, 2001; Laricchia, 2010; Moles and Westoby, 2004). In contrast others studies found that seed size was not an important factor affecting seed removal or predation by animals (e.g. Hua, 1997, Pizo, *et al*., 2006). Consequently, other seed characteristics may be involved in the selection of seeds by seed predators.

Low seed removal in the caged treatment supported the hypothesis that vertebrates are major seed removers. In addition, observations by camera trapping revealed that the most abundance potential seed removers were rats (*Rattus spp*.). This finding agreed with several studies in both forested and degraded areas (e.g. Wood and Elliott, 2003; Cramer *et al*., 2007; Fricke *et al*., 2014). In this study, the rats were speculated to remove seeds of the largest seeded species, *H. glabra,* in the cage treatment. The cages could not completely prevent *H. glabra* seeds from being removed and/or damaged. I found the evidence of digging around the corner of the cages. The successful removal of seeds around the edge of the cage by rats led to overestimate the seed removal in the cage treatments in *H. glabra*.

Figure 5.1 Evidence of seed removal and/or seed predation – a whole seed were removed from the bamboo tube (A), a seed was bitten by an animal (B), seeds were cracked and removed from the bamboo tube (C), and a seed was damaged by insects (D).

Insects were not major seed removers but damaged some seeds (Figure 5.1 D). Evidence of insect access to seeds included ant nests found inside a bamboo tubes and probably weevil holes on seed coats. Insecticide did not reduce seed removal. Although large number of insects, especially ants, was reduced little evidence of seed removal by insects was found. A previous study reported that ants are major seed predators for every seed species of a tropical savanna (Ferreira *et al*., 2011). In this study, the seeds of most of species were too large for insects to remove them away from the sowing location. However, some insects damaged the seeds by making a hole on seeds and consuming seed the materials such as cotyledons and embryo (Sallabanks and Courtney, 1992).

In this study, seed removal was used as a proxy to estimate the intensity of seed predation. To better clarify the fate of seeds, further studies should be done using different methods, for example, flagging seeds and releasing in the field. When seeds are taken, investigators can search for the flagged seeds and see if seeds are destroyed. In addition, the role of animals on seed removal can be confirmed using video trapping in the field.

5.2 Seed germination

Seed removers reduced the number of seed available for germination but did not affect the ability to germinate. The results of seed germination did not support the hypothesis that vertebrates and invertebrates reduce germinability. Species differed in seed germination and the exclusion treatments did not have significant effects on seed germination. Variations in seed germinability are influenced by intrinsic factors and environmental conditions. Internal factors include seed viability, seed characteristics (Amri, 2014), plant hormones (Miransari and Smith, 2014) and dormancy (Willoughby *et al*., 2004). External factors include environmental factors such as light, water availability, oxygen and temperature (Kyereh *et al*., 1999; Derroire, 2016). If the seeds are viable and seed dormancy is broken, germination is likely when the seeds are provided suitable conditions of light, moisture, temperature and oxygen.

Seed storage behavior and viability are primary factors that determine seed germinability. Seed storage behavior is classified into three groups (Hong and Ellis, 1996) – orthodox, recalcitrant and intermediate. According to whether seeds can be dried and how fast seeds loss viability after collection and during storage. In this study, *H. glabra* seeds were recalcitrant, which mean that they cannot be dried, without viability loss (Waiboonya, 2017). After collection, the viability of recalcitrant seeds declines over time (Mag'omba, *et al*., 2007). *H. glabra* seeds were collected and refrigerated at 4 °C for about two months before sowing. *H. glabra* seeds germinated in both the nursery and field experiments. Recalcitrant seeds must be sown immediately after collection to reduce seed germination loss.

A long period of seed dormancy contributes to the loss of vigor and viability (Debeaujon et al., 2000) and low germination (Pakkad, 2002). Two species *P. cerasoides* and *A. kurzii* had relatively rapid and high germination (two weeks after sowing). Rapid germination was positively associated with percent germination (Pakkad, 2002). In contrast, *H. dulcis* and *C. axillaris* took longer to germinate in the field. Previous studies reported that *C. axillaris* had no seed germination after one year in the direct seeding field trial because of long dormancy (Tunjai, 2005; Waiboonya, 2017). Furthermore, Brodie (2007) claimed that the germination *C. axillaris* seeds was influenced by animal dispersal. Consumption by animals allows seeds to pass through animals' digestive tracts resulting in reduced dormancy. Seed pretreatments to increase germination rate and percent germination, are necessary for species with long dormancy and low germination (FAO, 2017).

In addition, the main external factors influencing germination include soil moisture, temperature (Pakkard, 2002), oxygen, and light. In this study, soil moisture and oxygen may not be limiting factors for seed germination. The seeds were sown in the rainy season (209 – 330 mm in July and August) (see in Figure 3.2). In terms of light conditions, seed germination of many tropical tree species is associated with sunlight. *C. axillaris*, *P. cerasoides* and *A. kurzii*, seeds require high light conditions for germination (FORRU, 2006), whereas *H. dulcis* seeds better germinated in partial sunlight, at about 40% of full sun (Kopachon *et al*., 1996). In this study, *C. axillaris*, *P. cerasoides* and *A. kurzii* germinated equally well in the field and nursery. On the other hand, *H. dulcis* had lower germination in the field than in the nursery. The environmental conditions in the field may not be suitable for *H. dulcis* and may limit seed germination (Derroire, 2016). The germination response to environmental conditions varied among species and seed germination in the field must be tested for to select candidate species for direct seeding.

5.3 Cotyledonous-seedling and leafy-seedling mortality

The mortality of the cotyledonous-seedlings was lower than that of leafy-seedlings. The differences in mortality was due to differences in duration that seedlings stayed at each stage. The duration of cotyledonous-seedlings was short at about seven days in comparison to 300 days of leafy-seedling stage (experimental period). The long duration increased the probability of being attacked by herbivores and the probability of dying from stressful environmental conditions.

For the cotyledonous-seedlings, vertebrates were primary cause of seedling mortality, relatively to invertebrates. The cage treatments and the cage-plus-insecticide treatment reduced seedling mortality, but only insecticide application did not reduce the proportion of dead seedling. The results supported the hypothesis that vertebrates were the major cause of cotyledon-seedling mortality. Many studies in natural habitats have shown effects of vertebrate species on seedling mortality. Vertebrates eat cotyledons and/or young shoots (e.g. Wahungu *et al*., 2002; Bricker *et al*., 2010; Zhang *et al.*, 2017) (Figure 5.2). On the other hand, the finding that insecticide did not reduce seedling mortality was in contrast with a study in pine species. A previous study indicated that applying insecticide to seedlings increases seedling regeneration and survival (Rolando, 2006). The effects of vertebrates and invertebrates on seedling mortality varies among different ecosystems, according to the species present.

For leafy-seedlings, vertebrates and invertebrates were not major causes of mortality. Seedling mortality was not different among the treatments. In contrast with many studies (e.g. Meiners *et al*., 2000; Ferreira *et al*., 2011; Frick *et al*., 2014), my finding did not support the hypothesis that animals were a major cause of seedling mortality at this seedling stage. The intensity of seedling mortality by herbivores may differ among habitats, which have different herbivore communities.

The mortality of leafy-seedlings varied among species. The smallest seeded species in this study, *H. dulcis*, had the highest mortality (40% of mortality from total germination). Previous studies of direct seeding suggested that smaller seed species had lower success in seedling establishment in comparison with larger seeded species (Doust *et al*., 2006; Moles and Westoby, 2004; Tunjai and Elliott, 2012). Successful seedling establishment is associated with seed size (Coomes and Grubb, 2003, Doust *et al*., 2008, Muller-Landau 2010). In comparison to small seeds, large seeds have more seed reserves and usually produce large seedlings that have higher potential to tolerate poor light or low nutrient conditions (Coomes and Grubb, 2003). Moreover, large seeds can tolerate a variety of stresses and disturbances encountered during regeneration and have high competitive ability in high stressfulness sites (Coomes and Grubb, 2003; Muller-Landau 2010).

Competition with surrounding vegetation could be a major cause of leafy-seedling mortality (seedling height 8 - 48 cm). At the field site, herbaceous species were abundant; the dominant species were bracken fern (*Pteridium aquilinum*), blady grass (*Imperata cylindrical*), and green panicgrass (*Panicum maximum*). Although aboveground parts of the herbaceous species were removed five times during the study, the belowground parts were not removed so the herbaceous plants could re-grow. A high density of herbaceous plants may contribute to high belowground competition among seedlings and herbaceous plant roots (Douglas *et al.*, 2007; Doust *et al.*, 2008; Tielborger and Valleriani, 2005) and affect seedling survival (Figure 5.3).

Other factors including environmental conditions and plant pathogens can cause seedling mortality. In the study, the seeds were sown at the beginning of the rainy season but the seedlings grew through the dry season. Six percent of seedlings wilted and died. The conditions during seedling development may be unsuitable for individuals with low drought tolerance. In addition to dry conditions, seedling mortality can be caused by many plant pathogens such as fungi, bacteria and virus (Bel et al., 2006; Lindelow and Bjorkman, 2001; Waiboonya, 2017). The effect of plant pathogens on seedling mortality was beyond the scope of this study. Further studies are needed to determine whether plant pathogens limit successful establishment of small seedlings from the direct seeding method.

Figure 5.2 Evidence of seedling damage (A-D).

Figure 5.3 Ground herbaceous plants in the treatments contributed to inter-specific competition.

5.4 Seedling survival after the exclusion experiments were terminated

Seedling survival varied among tree species and is associated with seed size. The species were categorized into two groups of high and low survival. *P. cerasoides* and *C.axillaris* had high seedling survival (in agreement with Pakkad, 2002 and Waiboonya, 2017). The two species had medium size seeds in comparison with the other species in this study. This finding was similar to that Tunjai and Elliott (2011), who demonstrated that seedlings from medium- (0.1 - 4.99 g) to large- (> 5.0 g) seeded tree species had significantly high percent survival than small-seeded species (< 0.01 g). For the low survival species, *A. kurzii* and *H. dulcis* had relatively small seeds. The survival percentage was significantly lower than that of the first group with high survival. Previous studies indicated small seeded species had lower tolerance to harsh environmental conditions and lower competitive ability than larger seeded species (Doust *et al*., 2006; Pizo *et al*., 2006; St-Denis *et al*., 2013).

In addition, seedling characteristics influence seedling survivorship. Although this point was beyond the scope of this study, I hypothesized that seedling survival is associated with seedling morphology and physiology (Saverimuttu and Westoby, 1996). Various tree species in different genera have different seedling characteristics, such as leaf toughness, stem thickness and root morphology. The seedling characteristics are related to resource competition (Doust *et al*., 2008; Schreeg *et al.*, 2005), herbivore resistance (Barton and Hanley, 2013), and abilities to survive and grow under low resource availability (Beckage and Clark, 2003). Previous research studies showed that seedlings with larger root collar diameter and deeper root are most likely to survive and withstand in the face of animal disturbance and stressful conditions (Coomes and Grubb, 2003; Schreeg *et al*., 2005; Tsakaldimi *et al*., 2012,). Studies of seedling morphology in relation to tolerance to harsh environmental conditions and herbivory will help species selection for forest restoration. Selecting competitive stress tolerant tree species may ensure seedling survival. However, site maintenance including weeding and applying fertilizers to seedlings is still important to increase seedling survivorship (Fleury *et al*., 2015, FORRU 2006).

5.5 Relative growth rate (RGR) and species performance

The seedlings of the four studied species grew well in the field conditions. The final size of the 10-month old seedlings varied among species. On average, *P. cerasoides* seedlings grew the tallest with the widest crowns and thickest stems. However, the relative growth rates of all species were more than 100% per year in height, crown width and stem diameter. *P. cerasoides*, and *C. axillaris* grew five times taller per year. *A. kurzii* and *H. dulcis* grew slower but still grew taller more than three time per year. All four species expanded the crown two to five times per year. The four species have been reported to as fast growing species (FORRU, 2006) that grow rapidly under high light conditions (Goodale *et al*., 2014).

This study provided more information for species selection for direct seeding method. Previous studies suggested that suitable species for direct seeding should have high seed germination, high survival and high seedlings growth rate (Lamb, 2011; Tunjai and Elliott, 2011). In this study, the criterion of seed removal was taken into account in ranking the species. Among the studied species, the recommended species for direct seeding was *P. cerasoides.* The pioneer tree species, *P. cerasoides* provided the excellent performance rating score for direct seeding. *P. cerasoides* had rapid germination, low seed removal, high seedling survival and relative growth rate.

Two species with relatively high performance were *A. kurzii* and *C. axillaris*. Seed removal of the two species was low. However, these seed germination of *C. axillaris* and the survival of *A. kurzii* were also low. Further work, to increase percent seed germination and seedling survival will help improving their performance for direct seeding.

*H. dul*cis were not suitable for direct seeding. Although *H. dulcis* had low seed removal and high relative growth rate, this species had low seed germination and low seedling survival in the field. *H. dulcis* had better germination in nursery than in the field (in agreement with Waiboonya (2017)). This suggested that seedling production in the nursery and seedling plantation (seedlings of 30-50 cm tall) are more suitable for *H. dulcis*.

H. glabra was not suitable for direct seeding, For *H. glabra*, the challenges for direct seeding were high seed removal in the field and impossible long-term seed storage.

Previous research on this species found that sowing seeds immediately after collection increased germination, but percent seedling establishment was low at 10.9 ± 3.6 percent over one year (Waiboonya, 2017). The growth rate of *H. glabra* seedlings was low when compared with other species (Waiboonya, 2017). Overall, this species may be not suitable for direct seeding in degraded areas.

In practice, selecting species with no seed removal in the field is difficult. For direct seeding, restoration ecologists may find ways of protect seeds from being removed and/or damaged by vertebrates. The protection techniques include 1) seed coating with clays, 2) seed coating with animal deterrents, and 3) putting seeds in protective containers that are biodegradable in the field (Vaughan *et al*., 2017). Future studies are needed to develop techniques that are practical and suitable for different species.

5.6 Potential seed predators

a. Small mammals and birds

The animal species found in the studied site are rodents, birds, and small carnivores. *Rattus sp.* were most abundant Rodents are known to be seed predators of many plant species (e.g. Birkedal *et al*. 2010; Wood and Elliott, 2003; Doust *et al*., 2008) and barriers to successful direct seeding (Farlee, 2013). In this study found that one species in genus *Rattus* frequently visited the site, especially after seeds were sown. Photographs from the camera traps revealed that *Rattus* sp. usually searched inside the bamboo tubes. These observations, coupled with the findings of the previously mentioned studies, tend to suggest that rat is indeed an important seed predator.

Another potential seed predator species was the barred buttonquail (*Turnix suscitator*). They visited the site only shortly after seeds were sown, in August and September. The usual diet of barred buttonquails (*Turnix suscitator*) consists of grains and seeds (Arora, 2014). In this study, the barred buttonquail (*Turnix suscitator*) was categorized as a potential seed predator, based on their gape size and activity, as captured in the photos. The barred buttonquails were photographed searching and picking inside the bamboo tubes. However, this species is known to be omnivorous: they also eat mealworms (Arora, 2014).

In addition to potential seed predators, carnivorous species and potential seed dispersal agents visited the site. One individual each of leopard cat (*Prionailurus bengalensis*) and siamese jackal (*Canis aureus cruesemanni*) were also found in the study site. The presence of carnivorous species is usually correlated with that of their prey (Carbone and Gittleman, 2002). Leopard cats (*Prionailurus bengalensis*) are commonly found in open habitats, secondary forests and plantation areas as long as they have food (Sunquist *et al.*, 2007). Their diets include small mammals such as rat (Grassman, 2000), birds (Sunquist *et al.*, 2007), amphibians and reptiles. The diet and typical habitat of the siamese jackal are similar to those of the leopard cat (Borkowski *et al.,* 2011). Both siamese jackal and leopard cat are expected to be predator control population of seed predator and decrease the intensity of seed removal. Furthermore, various bird species perching on the ground were observed. It is worth noting that the study site is located 70 m away from the forest. It is possible that the species found with low frequency, including the leopard cat, Siamese jackal and some birds, may only have been at the site by chance.

b. Invertebrate species (insects)

Seventeen Orders of invertebrates were observed in the study (see Appendix C for pictures).The insects were divided to three groups, including seed predator, seedling predator and other invertebrates. For seed and seedling predators, many studies recognize both insect larva and adults as seed and seedling predators (e.g. Ferreira *et al*., 2011; Zhang *et al*, 1998).

For the seed predator group, some invertebrate family in Order Hymeoptera, Coleopteran and Hemiptera were categorized as seed predators. The most abundant was ant species (Order Hymenopter; Family Formicidae), which had chewing mouthparts. Ants can feed on seeds by bitten. They were reported as major seed predators of small seeds in degraded areas (Doust *et al*., 2008;Ferreira *et al*., 2011; Fricke *et al*., 2014).A study of direct seeding in abandoned agriculture lands of Northern Thailand found evidence of ant predation of small seeds (Wood and Elliott, 2003). Many ant species also act as seed dispersers (Hensen, 2002; Christianini and Oliveira, 2009). In this study, I observed ant nests in the control and the open cage treatments all species tested. I did not observe ants actually moving seeds. Therefore, the role of ants in seed removal and/or seed predation is inconclusive in this study. Coleopterans are generalist seed predators particularly of Fagacaea species (Pereira *et al*., 2014). In addition, Coleopterans bore into Leguminosae seeds, lay eggs inside the seeds and use seeds as larvae provision (Takakura, 2002).

The seedling predator group included species in Orders Coleoptera, Lepidoptera and Thysanoptera, Homoptera and Orthoptera. Among these Orders, Thysanoptera was the most abundant. They were suspected of chewing and sucking on seedlings (Zhang *et al*, 1998).Coleoptera species were classified as both seed and seedling feeders. To confirm that insect were classified in correct categories; seed or seedling feeder, insect should be determined in genus or species level.

The last group was other invertebrates with no evidence of being seed and/or seedling predators. Invertebrates in this category are beneficial in improving soil quality, controlling pest population (Gavloski, 2017). Invertebrates in Order Isoptera, Blattodea, Diptera, and Collembolla play roles in scavenging organic matters on the soil surface. Phasmatodea, Mantodea, Hemipter and Araneae are predators of other invertebrates. Classifying Diptera as non-seed predators was in contrast with a case study in Canada (Savage *et al.*, 2016). The study by Savage *et al.*, 2016 showed Diptera species were major pests of many vegetation crops. However, Diptera specimens collected from the field site had lapping mouthparts indicating they were scavenger (Vargas *et al.*, 2015). The references to Diptera species in this study could not be major seed or seedling predators.

5.7 Variation of animal visits and seed-seedling transitional stage

The number of vertebrate and invertebrate individuals visiting remained high during the seed stage and decreased after seedling emergence in October (Chapter 4, Table 4.2-4.3). Among vertebrates group, *Rattus sp*. was found in each month except December and January. For vertebrate group, seven species were detected only once; most of these were bird species. In December, the camera traps did not detect any small mammals or birds. For invertebrates group, the highest abundance was in rainy season and the abundance decreased in dry season.

The presence of animals in the areas depended on the animals' activities and movements. In this study, I did not test factors that affect the animal activities in the area. The temporal variation observed suggests the potential for future investigation. I suspect that animal activity patterns are affected by food supplies inside and outside the area and by climatic conditions (Geiser, 1987; Liu *et al*., 2013, Di Bitetti *et al*., 2008). Further studies are needed to determine the effects of seasonal and environmental conditions such as food abundance on the temporal variation in the presence of animals in the degraded area. The seed-to-seedling transitional stage is a critical period that determines plant distribution (Lewis and Gripenberg, 2008). For an application in direct seeding, understanding the dynamics of animals, in relation to plant stages, can be helpful to plan treatments to apply to seeds, to reduce seed and seedling loss by animals.

CHAPTER 6

Conclusions and Recommendations

6.1 Conclusions

1. The intensity of seed removal varied greatly, depending on seed characteristics, particularly seed size and ability of animal to access the sowing seeds. Tree species with seed size ranging from 0.024 to 4.247 g had average seed removal of 18.22 ± 2.64 percent. On the other hand, large seeded species, *H. glabra* suffered up to 100 percent seed removal, when the seeds were not protected from vertebrates.

2. Vertebrates played a major role in seed removal in comparison to insects. Camera trapping showed that rats (*Rattus* sp.) were the most abundant in the study site. The animal visits to study site peaked after seeds were sown. In addition to vertebrates, insect seed predators included ants (Order Hymenoptera) and insects in Order Coleoptera may also remove and/or damage seeds.

3. Seed germination varied among species and germinability was not affected by exposure to vertebrate and invertebrate species. In this study, *A. kurzii* and *P. cerasoides* had high percent germination in the field. All remaining seeds of *H. glabra* failed to germinate because of low viability before sowing. The variation in germination among species is influenced by seed characteristics that are associated with germination requirements.

4. In term of seedlings, average cotyledonous-seedling and leafy-seedling mortality across species were 4.62 and 21.40 percent, respectively. Caging seedlings significantly reduced percent mortality of cotyledonous-seedling. The finding indicated that vertebrates were the major cause of cotyledonous-seedling mortality. On the other hand, predator-exclusion did not reduce the mortality of leafy seedlings. The effects of herbivores on seedling mortality may differ among plant ontogenetic stages.

5. The study can be applied to species selection for direct seedling. The most excellent tree species was *P. cerasoides,* low seed predation, high percent germination, high seedling survival and relative growth rate. For two species, *A. kurzii*, *C. axillaris,* seed removal was low but they may require pretreatments to increase their ability to germinate and survive. Among studied species, *H. glabra* and *H. dulcis* were considered the least favorable for direct seeding. *H. glabra* had high seed predation and had recalcitrant seeds (See in Chapter 4, Table 4.5), while *H. dulcis* had low germination and low seedling establishment. The two species may be appropriate to germinate in the nursery and to use in the conventional tree planting method.

6.2 Recommendations

1. For direct seeding, site preparation should include surveying of potential seed predators before direct sowing. Different sites might have different types of natural enemies (Birkedal *et al*, 2010). A better understanding of species and animal dynamics in the area will guide appropriate site preparation and management plans.

2. To reduce the probability of seed predation, techniques to accelerate germination rate should be applied. Seed pretreatments may include removing seed coat or testa, seed soaking in acid or water, etc. before sowing seeds in the area. Selecting pretreatment methods depends on species and characteristics of seeds (Mng'omba, *et al*., 2007). Increasing of seed germination rate enhance plant survival and success rate on seedling establishment in the area (Tunjai and Elliott, 2012).

3. Protecting seeds from predators is necessary for large seeded species. Seed protection may be seed burial deeper in the soils (Doust *et al.*, 2006). In addition, seeds may be coated by clays or other materials and chemicals that help deter seed predators. An example could be urine of carnivores that prey on seed predators. The smell of carnivores may scare off seed predators. Selecting appropriate seed protection techniques for different species is an important topic for future research.

4. Direct seeding is appropriate for some tree species. For species that fail to establish by direct seeding method, conventional tree planting may be better for those species. Alternatively, a combination of direct seeding and conventional tree planting may be implemented.

5. The conservation carnivores will help to control populations of major seed predators, such as rats. For instance, the leopard cat is predator of small rodents.

6. For future research, the study of factors affecting seedling mortality will guide site management. Other natural enemies such as fungi and pathogen can be the cause of seed destroyed or seedling mortality. For conventional tree planting, seedlings were raised in tree nurseries and healthy seedlings are selected to plant in the target area. In comparison to conventional tree planting, small seedlings from direct seeded method may be more vulnerable to pathogens attack. If we have information about seedling losses due to different causes, we can estimate how many seeds need to be sown and whether additional treatments are necessary to prevent seedling mortality.

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APPENDICES

APPENDIX A

Plant species descriptions

Descriptions of each plant species in this study base on Gardner *et al.* (2007), The Botanical Garden Organization (2011), Pakkad (1997) and FORRU (2006). Plant scientific names, family names and local name follow The Plant List (2013) and Gardner *et al.* (2007). Seed volumes (width x long x thickness of seed) were measuring in this study.

***Hovenia dulcis* Thunb** **Mawn Hin(หมอนหิน)**

(RHAMNACEAE)

A large, pioneer, briefly deciduous tree, growing up to 30 m tall. This species were record as rare species in evergreen forest often along stream, seasonal, hardwood forestand open disturbed roadside, at elevation of 1,025 to 1,325 m above sea level.

Bark: thick bark with broad, longitudinal, grey or brown ridges, separated by narrow brick-red fissures (Figure 7.1A).

Leaf: spirally arranged, simple blade with ovate to elliptic (Figure 7.1B)

Flower: in cymes, numerous, light green and cream, small (March to May)

Fruit and seed: septicidal capsule, fruit stalks very thin and curving for 2-3 mm above each fruit, swollen and fleshy, green when fruit are unripe (Figure 7.1C), turning red-brown or black as fruit ripen(August to February), glossy, black seed per locule (4.60 x 4.84 x 2.13 $m^3$of seed volume), birds-dispersed particularly by pigeons (Kopachon *et al.*, 1996).

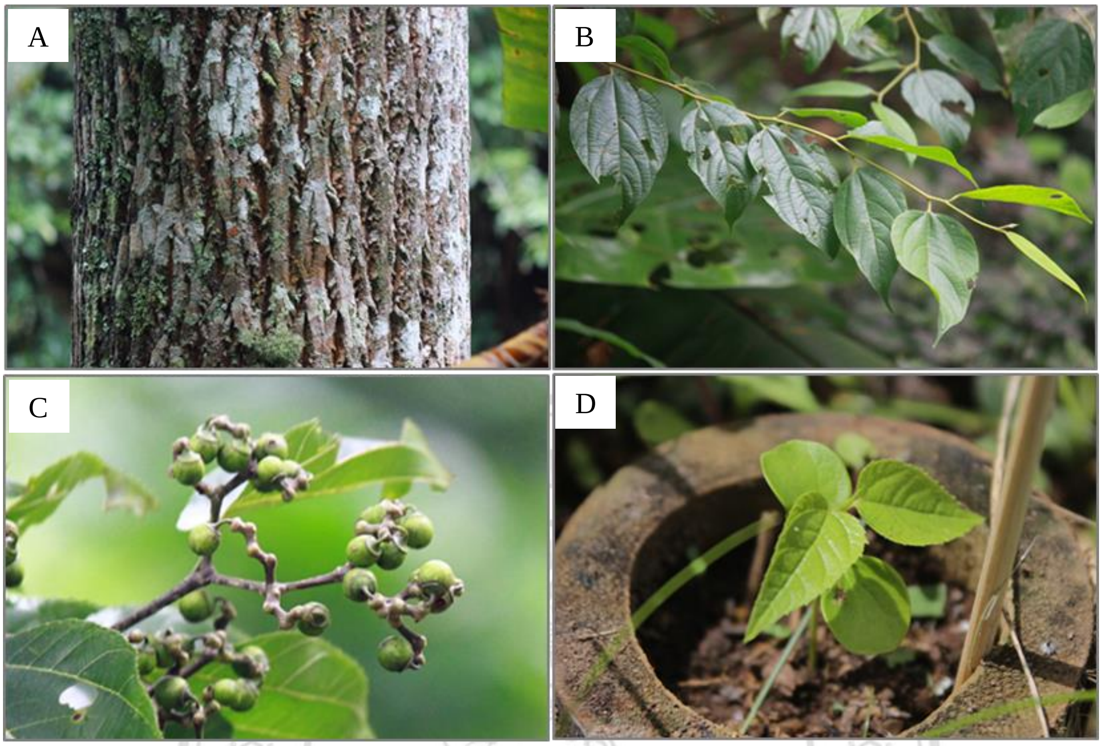


Figure 7.1 Bark (A), leaves (B), fruits (C) and small seedling (D) of *Hovenia dulcis* species.

***Alangium kurzii* Craib** **Sa Leek Dong (สะลีกดง)**

(CORNACEAE)

Pioneer tree, growing up to 28 m tall. Common in evergreen forest at elevation 600-1,400 m above sea level.

Bark: smooth, dark grey, lenticellate; inner bark orange and cream mottled (Figure 7.2A).

Leaf: broadly ovate with tapering tip and heart-shaped base, obviously asymmetric; mature leaves densely covered with soft golden hair below and on veins only above (Figure 7.2B).

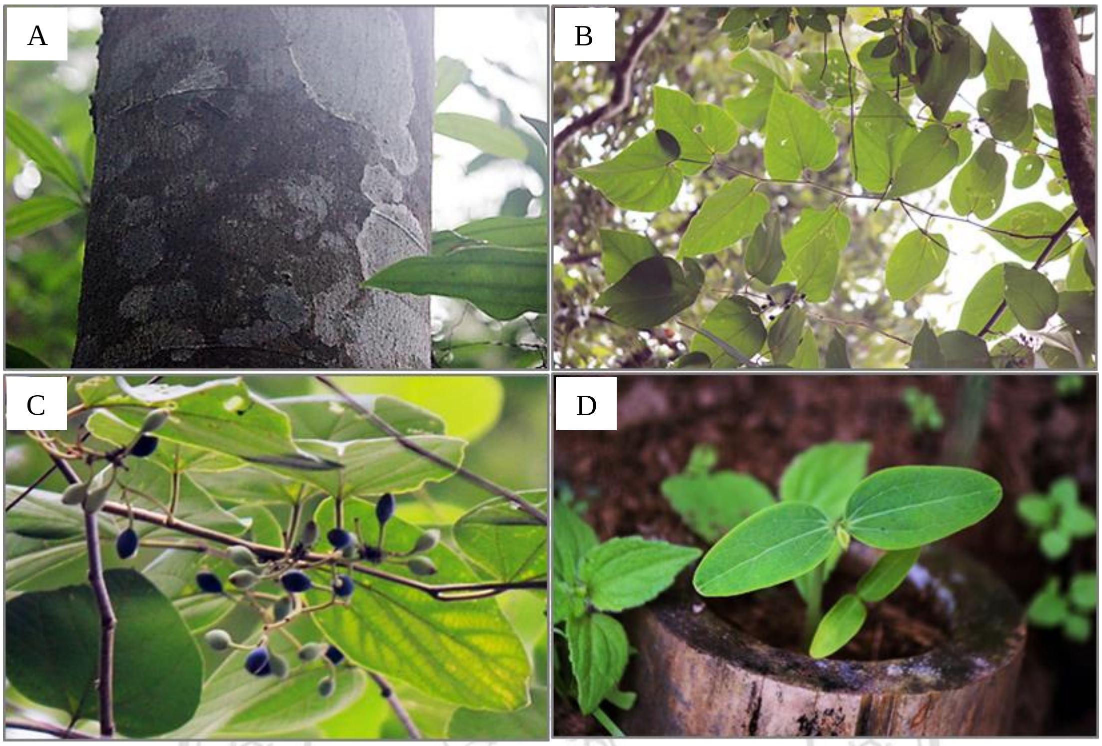


Figure 7.2 Bark (A), leaves (B), fruits (C) and small seedling (D) of *Alangium kurzii* species.

Flower: very fragrant, main stalks to 7-9 petals, dense silvery hairs, connectives also hairy (March to May).

Fruit and seed: 1.2-1.5 cm, ellipsoid with blunt tip, smooth to thinly hairy, sometime slightly grooved, crowned by distinct disc, ripening dark purple to black(June to September), contains one black seed (7.08 x 11.83 x 4.67 $m^3$of seed volume), oval with pointed ends (Figure 7.2C)., bird-dispersed

Prunus cerasoides D. Don Nang Paya Sua Krong (นางพญาเสือโคร่ง) (ROSACEAE)

A medium-sized, pioneer, deciduous tree, growing up to 16-18m tall. It's fairly common in evergreen forest, mixed-forest and evergreen forest-pine, often in disturbed areas, at elevations of 1,040 to 2,400 m above sea level.

Bark: shiny, red-brown, with large, raised, brown lenticels; outer layer peeling horizontally (Figure 7.3A).

Leaf: spirally arranged, simple, blades; margin finely serrate; dark red, stalked, glands where petiole meets blade (Figure 7.3B).

Flower: in axillary clusters, petals, pink; on leafless trees (December to January).

Fruit and seed: drupes (small cherries), ovoid, red when ripe, 10 -15 mm(March to May), each containing a single-seeded pyrene(7.31 x 9.67 x 6.01 m^{3}of seed volume); dispersed by birds, squirrels and other small mammals ((Figure 7.1C).

Birds such as, Sunbirds, Spider-hunters and White-eyes feed on the nectar, whilst bulbuls eat the fruits.

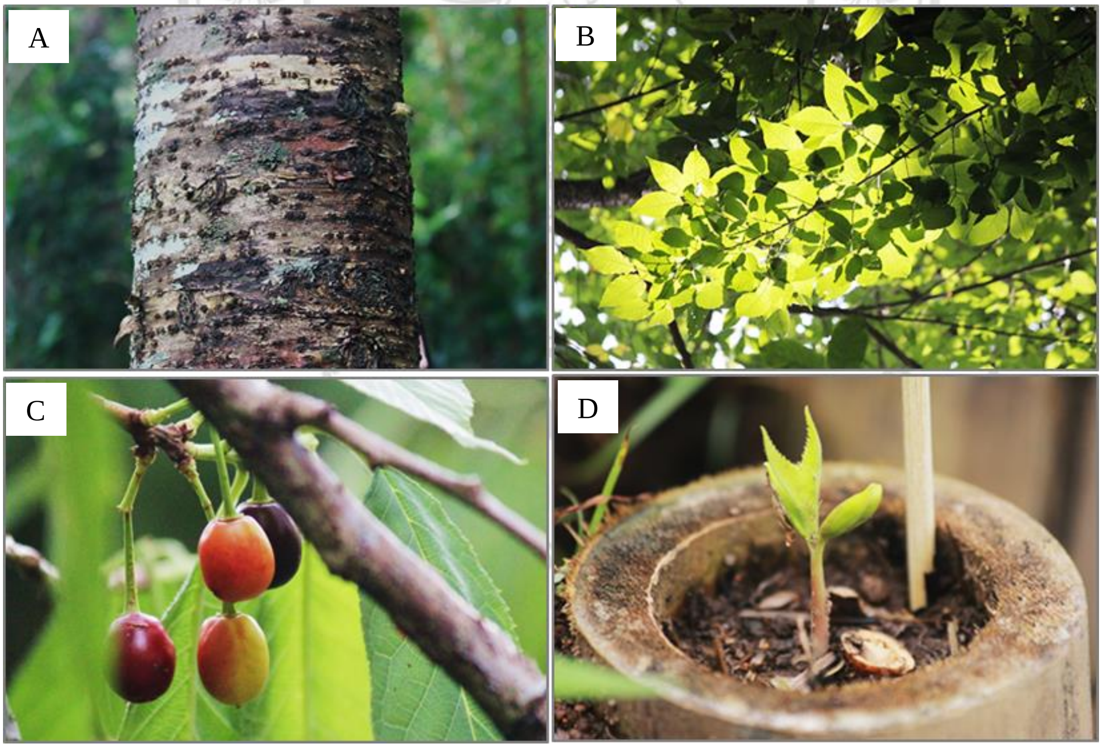


Figure 7.3 Bark (A), leaves (B), fruits (C) and small seedling (D) of *Prunus cerasoides* species.

***Choerospondias axillaris* Roxb.** **Ma Kak (มะกัก), Ma Mue (มะมือ)**

(ANACARDIACEAE)

A medium-sized, pioneer, deciduous tree, growing up to 25 to 30 m tall. The common plant species, widespread in evergreen forest, evergreen forest-pine and mixed forest at elevations of 700 to 1,600 m above sea level. Planted saplings achieve very high survival and growth rates. The tree support nesting birds from the 5th year after planting.

Bark: grey-brown, thin, vertically cracked (Figure 7.4A).

Leaf: spirally arranged, compound, once pinnate, leaflet blades opposite or sub-opposite, ovate to ovate-lanceolate, apex acuminate (Figure 7.4B).

Flower: male inflorescences 4-10 cm long; male corollas dark reddish purple, 0.4-0.5 cm; females solitary in upper leaf axils; January to March.

Figure 7.4 Bark (A), leaves (B), fruits (C) and small seedling (D) of *Choerospondias axillaris* species.

Fruit and seed: drupes, oval-shaped, with yellow leathery exocarp when ripe (June to August), 25-30 x 20mm across, each containing a single pyrene with 5 locules (13.81 x 18.83 x 13.67 m^3 of seed volume); animal-dispersed (fruits are eaten by deer, wild pigs and bears) (Figure 7.4C).

Horsfieldia glabra **(Reinw. ex Blume) Warb.** **Luead Ma (เลือดม้า)**

(MYRISTICACEAE)

Evergreen, climax, small to medium tree to 10 to 25 m. This species distributed or locally common in less-disturbed forest, seasonal, hardwood forest, granite bedrock, at elevation 200 to 1,060 m above sea level.

Figure 7.5 Bark (A), leaves (B), fruits (C) and small seedling (D) of *Horsfieldia glabra* species.

Bark: grey-brown, outer bark hard and brittle, inner bark yellow (Figure 7.5A).

Leaf: narrowly elliptic or obovate with blunt or pointed tip, smooth, dark green and glossy above (Figure 7.5B).

Flower: unisexual, pale yellow, in much-branched clusters at leaf axis or behind leaves; calyx globose or oval, often slightly triangular at base (September to October).

Fruit and seed: capsule fruit, yellow, smooth, firmly fleshy with single oblong seed (January to May), covered with thin orange coating (17.29 x 25.95 x 16.61m^3 of seed volume); animal-dispersed (Figure 7.5C).

APPENDIX B

Vertebrate and Invertebrate species

Photos from camera trap

Seed predators

Figure 7.6 *Rattus* sp. (Rat: หนูบ้านหรือหนูท้องขาว).

Figure 7.7 *Turnix suscitator* (Barred buttonquail: นกคุ่มอกลาย).

Non–Seed predators

Tree shrew species

Figure 7.8 *Tupaia belangeri* (Northern treeshrew: กระแตเหนือ).

Carnivore species

Figure 7.9 *Canis aureus cruesemanni* (Siamese jackal: หมาจิ้งจอกทอง).

Figure 7.10 *Prionailurus bengalensis* (Leopard cat: แมวดาว).

Figure 7.11 *Herpestes javanicus* (Small asian mongoose: พอนเล็ก หรือ พังพอนธรรมดา).

Figure 7.12 *Arctonyx collaris* (Hog badger:หมูหริ่ง).

Figure 7.13 *Viverra zibetha* (Large indian civet: ชะมดแผงหางปล้อง).

Bird species

Figure 7.14 *Anthus cervinus* (Red-throated Pipit: นกเด้าดินอกแดง).

Figure 7.15 *Centropus sinensis* (Greater coucal: นกกระปูดใหญ่).

Figure 7.16 *Lanius schach* (Long-tailed Shrike: นกอีเสือหัวดำ).

Figure 7.17 *Lonchura punctulata* (Scaly-breasted Munia: นกกระติ๊ดขี้หมู).

Figure 7.18 *Phylloscopus trochiloides* (Greenish Warbler: นกกระจิ๊ดเขียวคล้ำ).

Figure 7.19 *Pycnonotus aurigaster* (Sooty-headed bulbul: นกปรอดหัวสีเขม่า).

Figure 7.20 *Saxicola caprata* (Pied Bushchat: นกยอดหญ้าสีดำ).

<u>**Photos of Invertebrates species**</u>

A) Family Formicidae

B) Family Apidae C) Family Braconidae D) Family Ceraphronidae

E) Family Ichneumonidae

Mouthpart: Chewing

Feeder type: Herbivore, Carnivore and Omnivore

Figure 7.21 Example of insect in Order Hymenoptera (9 families, 3255 individuals).

A) Family Acrididae B) Family Tetrigidae

C) Family Gryllotalpa D) Family Gryllidae

Mouthpart: Chewing

Feeder type: Herbivore

Figure 7.22 Example of insect in Order Orthoptera (4 families, 62 individuals).

A) Family Chloropidae B) Family Dolichopolidae C) Family Muscidae

Mouthpart: Lapping

Feeder type: Scavenger

Figure 7.23 Example of insect in Order Diptera (12 families, 301 individuals).

Family Oligomuchinae

Mouthpart: Chewing

Feeder type: Predator

Figure 7.24 Example of insect in Order Mantodea (1 family, 1 individual).

Family Heteronemiidae

Mouthpart: Chewing

Feeder type: Herbivore

Figure 7.25 Example of insect in Order Phasmida (2 families, 2 individuals).

A) Family Aphididae B) Family Cicadellidae

Mouthpart: Sucking

Feeder type: Plant feeder

Figure 7.26 Example of insect in Order Homoptera (5 families, 36 individuals).

A) Larva of family Geometridae B) Larva of family Noctuidae

C) Larva of familyErebidae

Mouthpart: Chewing

Feeder type: Plant feeder

Figure 7.27 Example of insect in Order Lepidoptera (3 families, 49 individuals).

A) Family Pentatomidae B) Family Reduvidae C) Family Rhopalidae

D) Family Cyxiidae E) Family Miridae

Mouthpart: Sucking

Feeder type: Herbivore , Predator

Figure 7.28 Example of insect in Order Hemiptera (6 families, 14 individuals).

A) Family Carabidae

B) Family Chrysomelidae

C) Family Curculionidae

D) Family Scarabaeidae

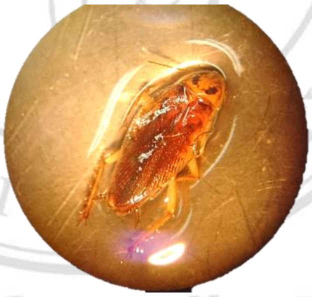
Mouthpart: Chewing

Feeder type: Predator, Herbivore

Figure 7.29 Example of insect in Order Coleoptera (9 families, 105 individuals).

A) Family Blatellidae

B) Family Blaberidae



Mouthpart: Chewing

Feeder type: Scavenger

Figure 7.30 Example of insect in order Blattodea (2 families, 8 individuals).

Family Forficulidae

Mouthpart: Chewing

Feeder type: Scavenger

Figure 7.31 Example of insect in Order Dermaptera (1 family, 2 individuals).

Family Termittidae

Mouthpart: Chewing

Feeder type: Scavenger

Figure 7.32 Example of insect in Order Isoptera (1 family, 3 individuals).

Family Corioxenidae

Mouthpart: Chewing

Feeder type: Parasitoids

Figure 7.33 Example of insect in Order Strepsiptera (1 family, 1 individual).

Springtail

Family Entomobryidae

Image from: https://extension.entm.purdue.edu/401Book/default.php?page=collembola

Mouthpart: Chewing

Feeder type: Scavenger

Figure 3.34 Example of insect in Order Collembola (1 family, 21 individuals).

Mouthpart: Chewing

Feeder type: Predator

Figure 3.35 Example of insect in Order Araneae (3 families, 3 individuals).

Mouthpart: Chewing

Habit: Herbivore

Figure 3.36 Example of insect in Order Gastropoda (1 family, 1 individual).

APPENDIX C

Statistic test

Seed removal model from GLM result

Call: glm(formula = cbind(Removed, (Sowing - Removed)) ~ Species + Treatment, family = binomial, data = Removal, weights = disp.weights)

Deviance Residuals:	Min	1Q	Median	3Q	Max
	-2.8261	-0.4561	-0.0707	0.2234	4.7994

Coefficients:	Estimate	Std. Error	z value	Pr(>|z|)
(Intercept)	-5.3404	1.1474	-4.654	3.25e-06 ***
SpeciesChoerospondias	1.3370	1.0023	1.334	0.182220
SpeciesHorsfieldia	10.5630	1.7850	5.918	3.26e-09 ***
SpeciesHovenia	0.5202	1.1212	0.464	0.642681
SpeciesPrunus	0.5202	1.1212	0.464	0.642681
TreatmentCage	-5.5826	1.6183	-3.450	0.000561 ***
TreatmentInsecticide	1.3612	0.8976	1.516	0.129408
TreatmentInsecticide+Cage	-3.1599	1.6424	-1.924	0.054358 .
TreatmentOpenCage	0.7438	0.9643	0.771	0.440510

--- Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

(Dispersion parameter for binomial family taken to be 1)

Null deviance: 801.287 on 74 degrees of freedom

Residual deviance: 58.748 on 66 degrees of freedom

AIC: 96.854 Number of Fisher Scoring iterations: 8

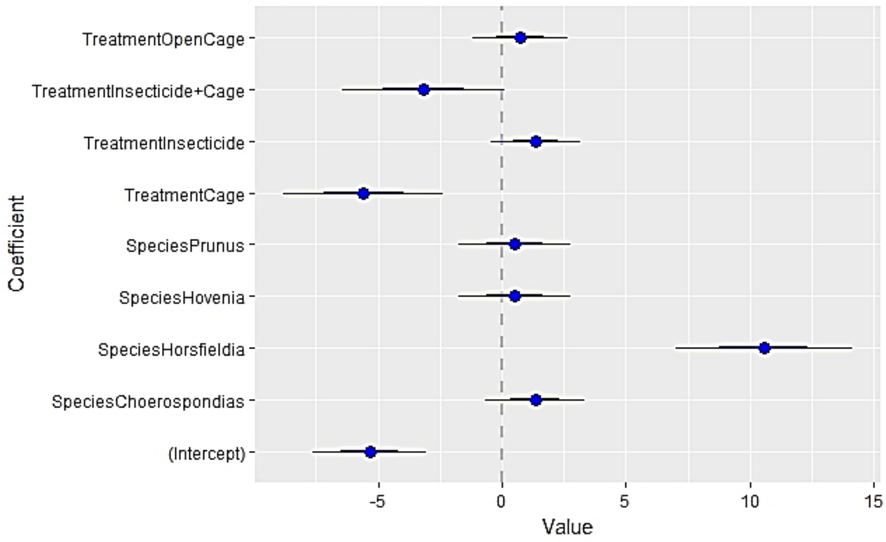


Figure 3.37 Coefficient plot of seed removal model from GLM.

Table 7.1 Probability of seed removal predicted by GLM.

	H. dulcis	*A. kurzii*	*P. cerasoides*	*C. axillaris*	*H. glabra*
Cage	0.00003	0.00002	0.00003	0.00007	0.41096
Insecticide	0.03050	0.01836	0.03050	0.06647	0.99862
Insecticide plus cage	0.00034	0.00020	0.00034	0.00077	0.88723
Open cage	0.01668	0.00998	0.01668	0.03698	0.99744
Control	0.00800	0.00477	0.00800	0.01793	0.99464

Relationship between seed removal and seed mass

Nonlinear regression model

Formula: Removal ~ exp(Mass * a) + 0

Parameters: Estimate Std. Error t value Pr(>|t|)

a 1.08079 0.01736 62.25 3.99e-07 ***

---Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 7.302 on 4 degrees of freedom

Number of iterations to convergence: 4

Achieved convergence tolerance: 6.178e-07

Seed germination model from GLM result

Call: glm(formula = cbind(Germination, (Sowing - Germination)) ~ Species, family = binomial, data = Germ, weights = disp.weights)

Deviance Residuals:	Min	1Q	Median	3Q	Max
	-3.2582	-0.6117	0.1900	0.6280	1.8579

Coefficients:	Estimate	Std. Error	z value	Pr(>|z|)
(Intercept)	1.01160	0.21243	4.762	1.92e-06 ***
SpeciesChoerospondias	-1.74502	0.29221	-5.972	2.35e-09 ***
SpeciesHovenia	-2.57379	0.32666	-7.879	3.30e-15 ***
SpeciesPrunus	-0.06714	0.29816	-0.225	0.822

---Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

(Dispersion parameter for binomial family taken to be 1)

Null deviance: 176.472 on 59 degrees of freedom

Residual deviance: 61.514 on 56 degrees of freedom

AIC: 118.93 Number of Fisher Scoring iterations: 5

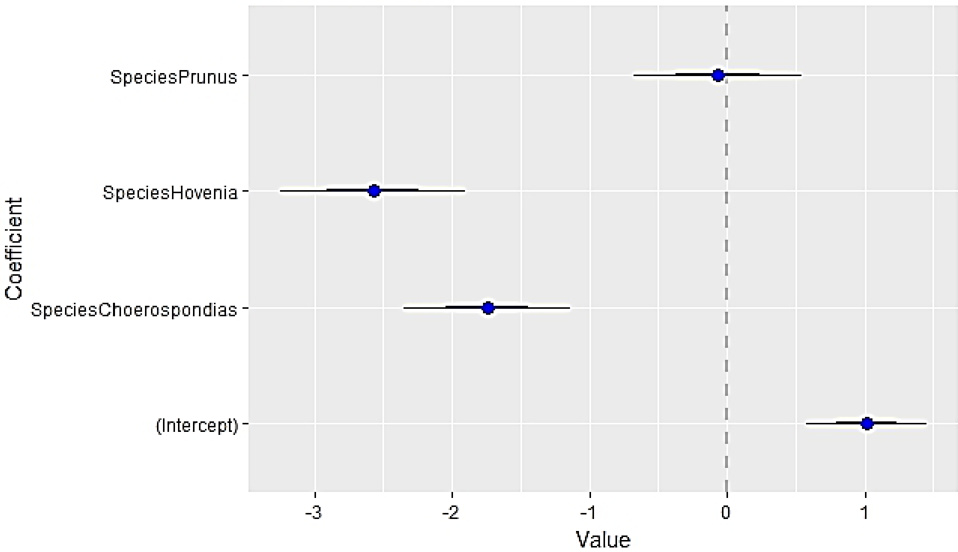


Figure 3.38 Coefficient plot of seed germination model from GLM.

######### When relevel and use Hovenia as a reference speciesChroerespondias has higher germiantion than Hovenia.

Call: glm(formula = cbind(Germination, (Sowing - Germination)) ~ Species, family = binomial, data = Germ2, weights = disp.weights)

Deviance Residuals:	Min	1Q	Median	3Q	Max
	-3.1642	-0.5941	0.1845	0.6099	1.8043

Coefficients:	Estimate	Std. Error	z value	Pr(>|z|)
(Intercept)	-1.5622	0.2555	-6.113	9.75e-10 ***
SpeciesAlangium	2.5738	0.3364	7.652	1.98e-14 ***
SpeciesChoerospondias	0.8288	0.3286	2.522	0.0117 *
SpeciesPrunus	2.5066	0.3342	7.500	6.39e-14 ***

---Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

(Dispersion parameter for binomial family taken to be 1)

Null deviance: 166.440 on 59 degrees of freedom

Residual deviance: 58.017 on 56 degrees of freedom

AIC: 112.62 Number of Fisher Scoring iterations: 5

Table 7.2 Percent probability of seed germination predicted from GLM model.

H. dulcis	***A. kurzii***	***P. cerasoides***	***C. axillaris***
17.33	73.33	72.00	32.44

Cotyledonous-seedling mortality from GLM result

Call: glm(formula = cbind(Mortality$Cmortality, (Mortality$Germination - Mortality$Cmortality)) ~ Species + Treatment, family = binomial, data = Mortality)

Deviance Residuals:	Min	1Q	Median	3Q	Max
	-1.7905	-0.7019	-0.3748	0.4017	2.0005

Coefficients:	Estimate	Std. Error	z value	Pr(>|z|)
(Intercept)	-2.3953	0.3536	-6.774	1.26e-11 ***
SpeciesChoerospondias	-0.9762	0.5673	-1.721	0.08529 .
SpeciesHovenia	-1.7945	1.0408	-1.724	0.08468 .
SpeciesPrunus	0.2251	0.3272	0.688	0.49138
TreatmentCage	-1.7292	0.6565	-2.634	0.00844 **
TreatmentInsecticide	0.6387	0.3917	1.631	0.10293
TreatmentInsecticide+Cage	-1.3592	0.5897	-2.305	0.02116 *
TreatmentOpenCage	-0.4584	0.4583	-1.000	0.31719

---Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

(Dispersion parameter for binomial family taken to be 1)

Null deviance: 75.667 on 56 degrees of freedom

Residual deviance: 39.472 on 49 degrees of freedom

AIC: 114.47 Number of Fisher Scoring iterations: 6

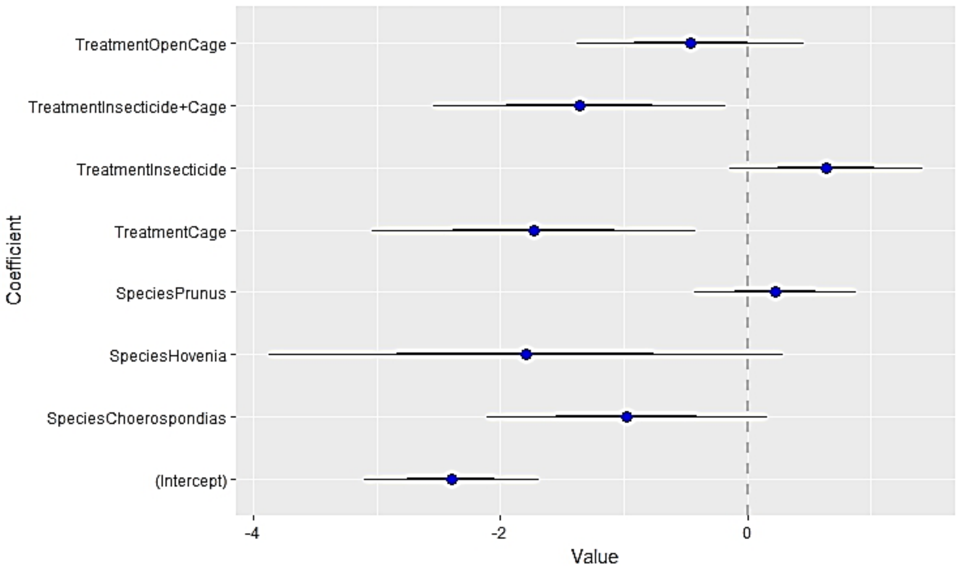


Figure 3.39 Coefficient plot of Cotyledonous-seedling mortality model from GLM.

Table 7.3 Probability of Cotyledonous-seedling mortality from GLM prediction model.

	H.dulcis	*A. kurzii*	*P. cerasoides*	*C. axillris*
Control	0.015	0.084	0.102	0.033
Cage	0.003	0.016	0.020	0.006
Insecticide	0.028	0.147	0.178	0.061
Insecticide plus cage	0.004	0.023	0.028	0.009
Open cage	0.009	0.054	0.067	0.021

Leafy-seedling mortality

Call:glm(formula = cbind(Lmortality$Dead, (Lmortality$Germination - Lmortality$Dead)) ~ Species + Treatment, family = binomial, data = Lmortality, weights = disp.weights)

Deviance Residuals: Min 1Q Median 3Q Max

-2.2678 -0.7980 -0.1651 0.7246 2.6816

Coefficients:	Estimate	Std. Error	z value	Pr(>|z|)
(Intercept)	-1.32295	0.36302	-3.644	0.000268 ***
SpeciesChaerospondias	-0.28853	0.40518	-0.712	0.476392

SpeciesHovenia	1.09053	0.38276	2.849	0.004384 **
SpeciesPrunus	0.02046	0.34468	0.059	0.952662
TreatmentCages	-0.85329	0.45187	-1.888	0.058979.
TreatmentInsecticide	0.11985	0.39119	0.306	0.759314
TreatmentInsecticide+Cages	-0.32137	0.40467	-0.794	0.427096
TreatmentOpen	-0.63058	0.43571	-1.447	0.147823

---Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

(Dispersion parameter for binomial family taken to be 1)

Null deviance: 79.263 on 56 degrees of freedom

Residual deviance: 58.554 on 49 degrees of freedom

AIC: 128.3   Number of Fisher Scoring iterations: 4

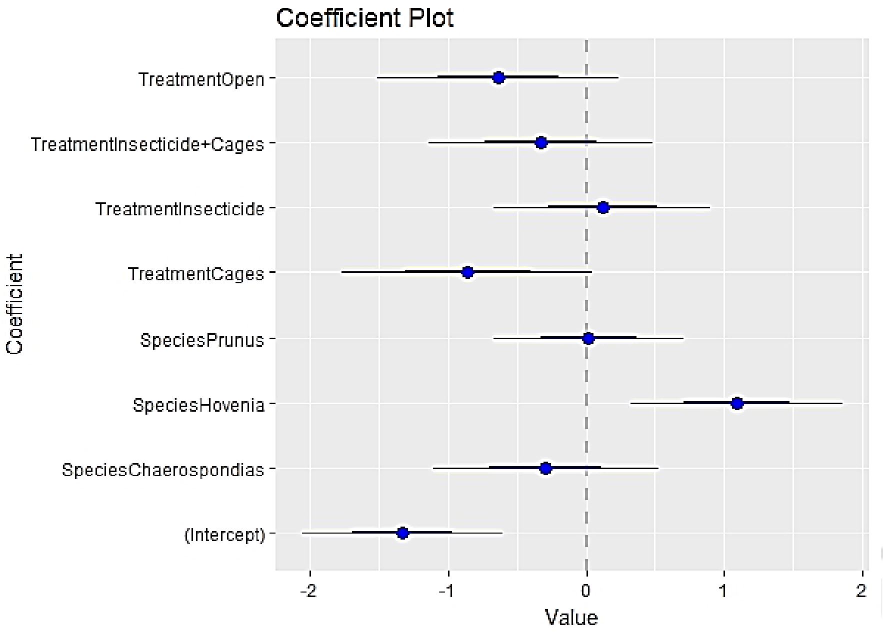


Figure 3.40 Coefficient plot of leafy-seedling mortality model from GLM.

Table 7.4 Probability of leafy-seedling mortality from GLM prediction model.

	H.dulcis	*A. kurzii*	*P. cerasoides*	*C. axillris*
Control	0.442	0.210	0.214	0.166
Cage	0.252	0.102	0.104	0.078
Insecticide	0.472	0.231	0.235	0.184
Insecticide plus cage	0.365	0.162	0.165	0.126
Open cage	0.297	0.124	0.126	0.096

T-test <u>-> test between mortality per day of cotyledonous-seedling and leafy-seedling</u>

Alternative hypothesis: true difference in means is not equal to 0

***H. dulcis*:** t = -2.4004, df = 4, p-value = 0.07432

95 percent confidence interval: -0.85403771 0.06203771

sample estimates:mean of x mean of y

0.000 0.396

A.kurzii: t = 2.6743, df = 5.1757, p-value = 0.04261*

95 percent confidence interval: 0.0444665 1.7888668

sample estimates:mean of x mean of y

1.0383333 0.1216667

P. cerasoides: t = 2.9783, df = 5.0153, p-value = 0.03074*

95 percent confidence interval: 0.1638399 2.2161601

sample estimates:mean of x mean of y

1.3033333 0.1133333

C. axillaris: t = 0.6839, df = 5.2471, p-value = 0.5231

95 percent confidence interval: -0.4465007 0.7765007

sample estimates: mean of x mean of y

0.23833333 0.07333333

<u>Seedling survival</u>

glm(formula = cbind(Survive, (Germinated - Survive)) ~ Species, family = binomial, data = Survival, weights = disp.weights)

Deviance Residuals: Min 1Q Median 3Q Max

-1.44052 -0.62329 -0.09298 0.56284 1.73860

Coefficients: Estimate Std. Error z value Pr(>|z|)

(Intercept) 0.3645 0.3314 1.100 0.27151

SpeciesAlangium -1.5924 0.5111 -3.116 0.00184 **

SpeciesChoerospondias -0.2404 0.4813 -0.500 0.61742

SpeciesHovenia -1.6331 0.5734 -2.848 0.00440 **

---Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

(Dispersion parameter for binomial family taken to be 1)

Null deviance: 24.9167 on 11 degrees of freedom

Residual deviance: 8.0787 on 8 degrees of freedom

AIC: 25.58 Number of Fisher Scoring iterations: 4

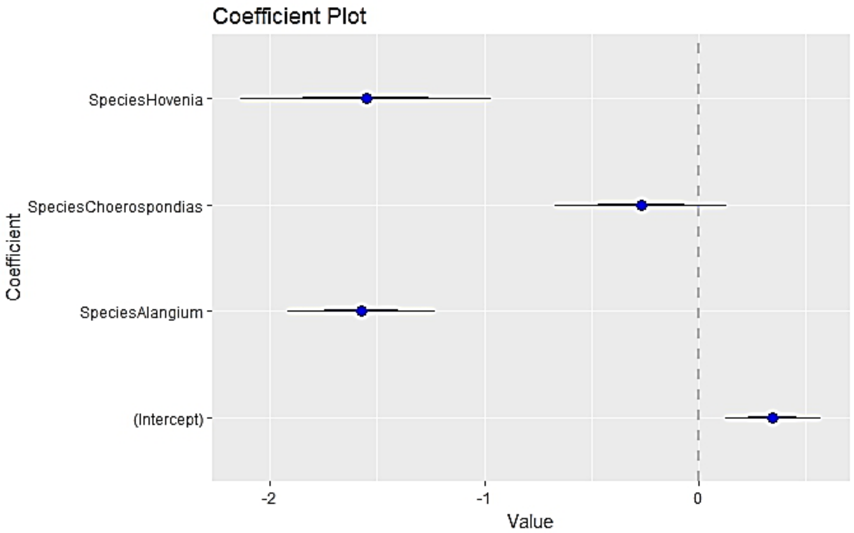


Figure 3.41 Coefficient plot of seedling survival model from GLM.

Table 7.5 Percent probability of seedling survival predicted from GLM model.

H. dulcis	***A. kurzii***	***P. cerasoides***	***C. axillaris***
22.0	23.0	58.0	52.0

APPENDIX D

Insecticide application

Trade name: Kino505

Common name: Chlorpyrifos and Cypermethrin

Chemical: Organophosphate compound and Pyrethroid compound

Targets: Insect such as aphids, caterpillar, ants and termites

Cypermethrin kills insects that eat or come into contact with it. This chemical works by quickly affecting the insect's central nervous system. The typical half-life of cypermethrin in the soil is 30 days and it can be rapidly broke down by soil microbes. Cypermethrin is highly toxic to fish, birds, bees and other insects. People handling or working with cypermethrin sometimes developed tingling, burning, dizziness and itching (NPIC, 1998).

Chlorpyrifos works by blocking an enzyme which controls messages that travel between nerve cells. Chlorpyrifos affects the nervous system of people, pets, and other animals the same way it affects the target pest. When chlorpyrifos released into the soil, it can take weeks to years for all of the chlorpyrifos to break down by ultraviolet light and chemicals in the soil (NPIC, 2010)

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