

## CHAPTER 5

### Discussions

#### 5.1 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; Tsakalidimi *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. dulcis* 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  $10.9 \pm 3.6$  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 Hymenoptera, Coleopteran and Hemiptera were categorized as seed predators. The most abundant was ant species (Order Hymenoptera; 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 Fagaceae 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.