Research Proposal

Research Grant for New Scholar

Part 1

Project title	Direct seeding techniques and aerial seed delivery for forest restoration of degraded areas in Northern Thailand
Project duration	2 years (24 months)
Total budget	600,000 Thai baht
Principal Investigator	Dr Pimonrat Tiansawat / Department of Biology, Faculty of Science, Chiang Mai University

Abstract

Forests are important for maintaining biodiversity and mitigating climate change. In its 20-Year National Strategy Framework, Thailand has set a target of increasing forest area to 40% of land area. Direct seeding is a method in which seeds are sown directly on the ground, and is a promising technique for forest restoration on a large scale. The challenges for direct seeding are to minimize seed loss after sowing and to promote seed germination and seedling growth. Therefore, determining practical techniques for protecting seeds and encouraging seed germination and seedling growth is a priority for direct seeding. In the first year of the proposed study, seeds of five native tree species, collected from natural forests, will be directly sown in two degraded areas in Chiang Mai with seven seed coating and enclosing treatments. Seed removal, germination, and seedling survival and growth will be monitored. Parallel tests of seed treatments will be done in a tree nursery. After the seed treatments are determined, it is necessary to develop efficient means to deliver seeds to the sites. One approach is to deliver seeds from the air (aerial seeding) by unmanned aerial vehicles (UAVs). In the first year, delivery systems will be designed and built, which will consist of a seed container and a release mechanism. In the second year, the two best seed treatments from the first-year direct seeding will be used in testing aerial seeding by a UAV at one of the degraded areas. Finally, seed and seedling data collected from direct seeding and aerial seeding, along with seed and seedling traits and site environmental factors will be analyzed. The proposed study will provide guidelines for implementing direct seeding for forest restoration. This study will integrate forest restoration science with modern UAV technology, with the aim of increasing the forest area of the country.

Keywords: Ecological restoration, seed traits, seed germination, seedling growth, drone

Research area/Sub area of this project: Ecology/ restoration ecology

Part 2 Proposal

1. **Project title** Direct seeding techniques and aerial seed delivery for forest restoration of degraded areas in Northern Thailand

2. **Principal investigator**

   2.1 Name Dr Pimonrat Tiansawat / 35 years old

   2.2 Position Lecturer Dr / Salary: 50,621 Baht

   2.3 Education Doctor of Philosophy in Plant Biology, University of Illinois, USA (Thesis: Ecological Significance of Seed Traits in the genus *Macaranga*)

   2.4 Department of Biology/ Faculty of Science/ Chiang Mai University/ 239 Huaykeaw Road, Suthep, Muang, Chiang Mai 50200

   2.5 Telephone 053-943346 (Work), 085-520-9388 (Mobile)
   Email address: tiansawat@yahoo.co.th, pimonrat.t@cmu.ac.th

   2.6 Weekly hours intended to spend on this project is 18 hr.

3. **Mentor**

   3.1 Dr Stephen Elliott

   3.2 Academic position Lecturer Dr

   3.3 Institute / Address Department of Biology, Faculty of Science, Chiang Mai University 239 Huaykeaw Road, Suthep, Muang, Chiang Mai 50200

   3.4 Academic field of specialty: Restoration ecology (tropical forests)

   3.5 Examples of recent research outputs (Since 2012)

1. Sangsupan, H.A., D.E. Hibbs, B.A. Withrow-Robinson, **S. Elliott.** 2018. Seed and microsite limitations of large-seeded, zoochorous trees in tropical forest restoration plantations in northern Thailand. Forest Ecology and Management 419-420: 91-100.
2. Caughlin, T.T., **S. Elliott**, J.W. Lichstein. 2016. When does seed limitation matter for scaling up reforestation from patches to landscapes? Ecological Applications 26:2439-2450.
3. **Elliott, S.** 2016. The potential for automating assisted natural regeneration of tropical forest ecosystems. Biotropica 48:825-833.

4. Kavinchan, N., P. Wangpakapattanawong, **S. Elliott,** S. Chairuangsri, J. Pinthong. 2015. Use of the framework species method to restore carbon flow via litterfall and decomposition in an evergreen tropical forest ecosystem, Northern Thailand. Kasetsart Journal - Natural Science 49:639-650.
5. **Elliott, S. D**, D. Blakesley, and K. Hardwick. 2013. Restoring Tropical Forests: A practical guide. Kew Publishing, 331 pages, ISBN 978-1-84246-442-7.
6. Hardwick, K. A., P. Fiedler, L. C. Lee, B. Pavlik, R. J. Hobbs, J. Aronson, M. Bidartondo, E. Black, D. Coates, M. I. Daws, K. Dixon, **S. Elliott**, K. Ewing, G. Gann, D. Gibbons, J. Gratzfeld, M. Hamilton, D. Hardman, J. Harris, P. M. Holmes, M. Jones, D. Mabberley, A. Mackenzie, C. Magdalena, R. Marrs, W. Milliken, A. Mills, E. N. Lughadha, M. Ramsay, P. Smith, N. Taylor, C. Trivedi, M. Way, O. Whaley, and S. D. Hopper. 2011. The Role of Botanic Gardens in the Science and Practice of Ecological Restoration. Conservation Biology 25:265-275.
7. Kuaraksa, C., and **S. Elliott**. 2013. The Use of Asian *Ficus* Species for Restoring Tropical Forest Ecosystems. Restoration Ecology 21:86-95.
8. Kuaraksa, C., **S. Elliott**, and M. Hossaert-Mckey. 2012. The phenology of dioecious *Ficus* spp. tree species and its importance for forest restoration projects. Forest Ecology and Management 265:82-93.
9. Tunjai, P., and **S. Elliott**. 2012. Effects of seed traits on the success of direct seeding for restoring southern Thailand's lowland evergreen forest ecosystem. New Forests 43:319-333.

4. Introduction to the research problem and its significance

Forest restoration is one of the most important tasks for recovering biodiversity and mitigating the global climate crisis. Forests provide habitats for plants and animals. In addition, plants absorb carbon dioxide (CO_2), a main driver of global climate change, and use it in photosynthesis. As a consequence, restoring vegetation in degraded areas increases habitat and food resources and reduces the amount of atmospheric CO_2. In recognition of the importance of forests, Thailand has set a strategic goal to increase forest area to 40% of the country's area, or approximately 205,000 km^2, by 2037. However, most forest restoration methods are only possible on a small scale because conventional tree planting is cost and labor intensive, as it involves the production of seedlings and the transportation of heavy seedlings for planting in degraded areas. Therefore, the Thai people face a new challenge to find techniques for restoring forests on a large scale to meet the country's goal.

An alternative method called direct seeding involves sowing seeds of forest tree species directly on the ground of degraded areas. The direct seeding method costs less than conventional planting methods because it does not require the establishment cost of tree nurseries and it is less labor intensive in seedling preparation (Harris and Leiser 1979, Wood and Elliott 2004). In addition, trees from direct seeding have higher growth rate in comparison to conventionally planted seedlings (Naruangsri 2017). However, seed removal and low germination percentage are challenges for direct seeding and delay the success of seedling establishment in degraded areas (Naruangsri 2017).

To ensure success in direct seeding, it is a high priority to develop techniques to reduce seed removal and increase germination percentage and germination rate. Previous studies have shown that rodents are the most common seed predators in degraded areas (Fricke *et al*. 2014; Wood and Elliott 2004, Naruangsri and Tiansawat 2016; Naruangsri 2017). Two approaches for overcoming the seed removal/predation problem are (1) to enclose seeds with protective materials and (2) to promote seed germination and early seedling growth by coating seeds with beneficial substances. The knowledge gained from this proposed study will be used as a guideline for seed handling and for implementing direct seeding for forest restoration in Thailand.

For restoration on a large scale, unmanned aerial vehicles (UAV) are promising in their ability to deliver seeds to degraded areas. Most areas targeted for forest restoration are remote and have rough terrain, difficult for humans to access. Aerial seeding, the combination of suitable seed handling techniques and UAV technology, has the potential to become a reliable option to overcome these difficulties. Aerial forest restoration has been used in other countries (Raffaella et al. 2015) but there have been few attempts in Thailand (Goldapple 2017). However, there is no record of seedling survival and growth rates after seeds have been delivered. This proposed study will be the first in Thailand to determine seed enhancement techniques, to build UAV seed delivery systems, and to monitor seedling performance after aerial delivery (Figure 1).

In this research study, there are five main research questions.

1. What method is efficient for reducing seed removal on the ground of degraded areas?
2. What coating substances increase seed germination rate and percentage for seeds sown on the ground of degraded areas?

3. What characteristics of seeds and seedlings contribute to fast seedling growth and high survival in degraded areas of Northern Thailand?
4. To what extent do site conditions affect seed germination and seedling survival and growth?
5. To what extent does aerial seeding (i.e. seeds delivered by a UAV) affect seed germination and seedling survival in degraded areas?

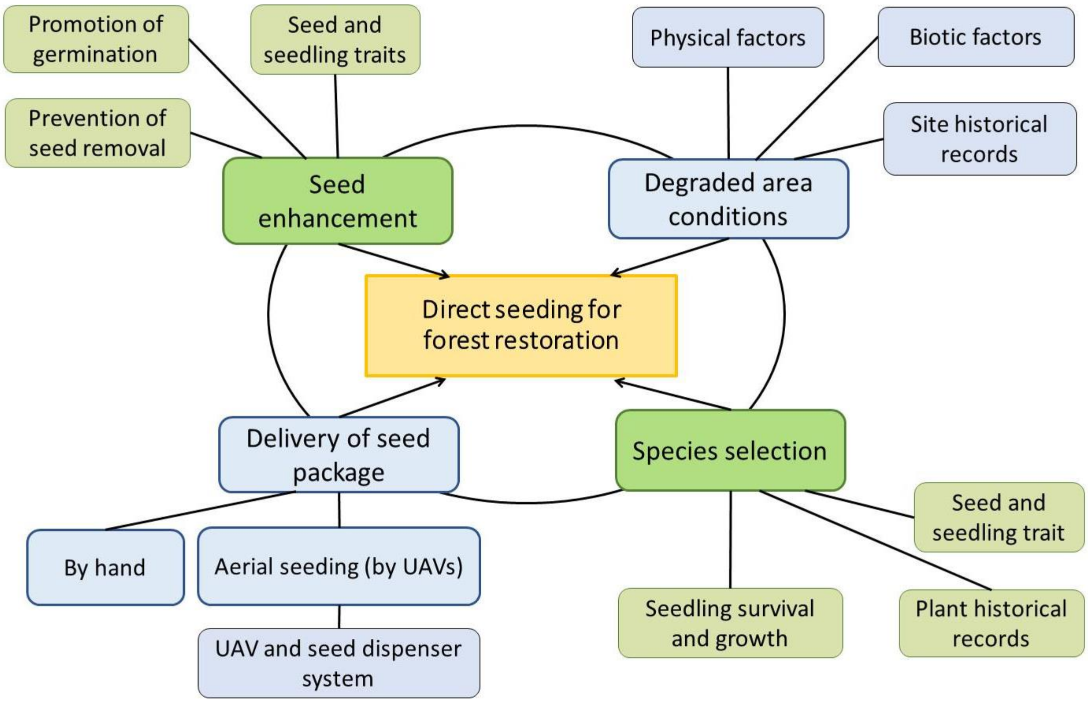

Figure 1 Conceptual framework of this proposed study

5. Literature review

Direct seeding for forest restoration involves sowing or burying seeds of tree species directly on the ground in areas needing to be restored. In direct seeding, people are the dispersal agents, bringing tree seeds to degraded areas where natural seed dispersal is limited. One advantage of direct seeding is that the space and costs associated with constructing tree nurseries are not required. In addition, the labor and time needed to produce large-enough seedlings in tree nurseries are eliminated (Cole et al. 2011). Therefore, the cost of establishing a mixture of native tree species in degraded areas by direct seeding can be cheaper than planting seedlings that are grown in tree nurseries (Harris and Leiser 1979, Wood and Elliott 2004). Moreover, seedlings from direct seeding have higher relative growth rate than those grown in nurseries that are transplanted in the same area (Naruangsri 2017).

Some studies have tested the potential of direct seeding in Thailand (e.g. Wood and Elliott 2004, Tunjai 2005; Tunjai and Elliott 2012; Hossain et al. 2014; Waiboonya 2017, Naruangsri 2017). In Southern Thailand, Tunjai and Elliott (2012) suggested that seed size, shape and moisture content are correlated with successful germination. Large seeds (> 5 g) with round shape and thick seed coat (> 0.4 mm) are likely to be suitable for direct seeding. In Northern Thailand, direct seeding was used to restore a lowland deciduous forest from bare land at a lignite mine (Hossain et al. 2014). Hossain et al. (2014) suggested two medium-to-large-seeded species, *Schleichera oleosa* (seed mass 0.5 g) and *Afzelia xylocarpa* (seed mass 5.5 g) for mine rehabilitation. However, studies in degraded areas nearby a natural montane forest in Northern Thailand have reported seed loss (up to 100%) due to animals. Seed loss is more severe in large seeded species than in smaller seeded ones (Waiboonya pers comm., Naruangsri 2017).

There are challenges for direct seeding in tropical habitats. External factors play a role in successful seedling establishment in degraded areas. These factors include physical conditions of the site (Doust et al. 2008), competition with weeds (Douglas *et al*., 2007; Doust et al. 2008), and seed and seedling loss due to predation by animals (Hau 1997, Wood and Elliott 2004, Orrock et al., 2006, Fricke et al., 2014; Naruangsri 2017). In degraded areas, dry conditions can limit seed germination and seedling growth (Budelsky and Galatowitsch 1999). In addition, herbaceous weeds grow quickly and suppress growth of small seedlings. Moreover, animal seed predators can significantly reduce the number of seeds available for regeneration. Previous works have shown that in degraded areas rodents are the most common seed predators, particularly for large seeded species (Wood and Elliott 2003, Fricke *et al*. 2014; Naruangsri and Tiansawat 2016; Naruangsri 2017). Therefore, it is necessary to find out suitable techniques to reduce seed predation, and to promote seed germination and seedling growth to ensure success of direct seeding for forest restoration.

Seed coating is expected to be a practical method to promote seedling establishment in direct seeding. Seed coating refers to the process of applying materials to the seed surface (Pedrini et al. 2017). The benefits of seed coating are adding weight to increase the size of very small seeds, preventing seed and seedling predation, and preventing diseases (Germains seed technology 2018, Pedrini et al. 2017). Currently, seed coating techniques are widely used in crop plants to enhance seed germination and support small seedling growth under unsuitable conditions (Mei *et al*. 2017).

There are several coating techniques being used. Asch (2014) reported that coating cereal seeds by water absorbent materials helped to promote seed germination and growth of early seedlings. Similarly, early seedling survival of a legume species in drought areas is higher for seeds that are coated with water absorbent polymers in comparison to uncoated seeds (Su et al. 2017). Biochar is another material that has been used to promote seed germination and seedling growth. Biochar is charcoal made by burning plant biomass at high temperature (e.g. 200-300 °C) in an inert atmosphere. Biochar can be used to improve soil conditions and increase water retention for seed germination and seedling growth (Solaiman et al. 2012). In addition, coating seeds with plant-growth-promoting microorganisms increases seedling growth of crops. Rice seeds mixed with *Streptomyces thermocarboxydus* (actinobacteria associated with spores of arbuscular mycorrhizal *Funneliformis mosseae*) grow better in low nutrient soil under drought stress (Lasudee et al. 2018). In some cases, seeds can be coated with agrochemicals, such as fungicides, insecticides, and pesticides to reduce seed predation and diseases (Pedrini et al. 2017). In forest restoration, seed coating is a new approach and the effects of coating materials on forest seeds are still unexplored.

Protecting seeds from animal seed predators is also necessary. In degraded areas where rodents are major seed predators, installing wire cages significantly reduces the probability of seed removal (Naruangsri 2017; Reque and Marti 2015). However, caging all seeds is impractical for direct seeding on a large scale. One realistic solution is to enclose seeds with protectant materials, such as soil particles or soil mixture, to form a spherical shape (Ortolani et al. 2015; Grossnickle and Ivetić 2017). The seeds together with their enclosing materials are usually called ‘seed balls’ or ‘seed bombs’. The enclosing technique has been tested in many countries including Kenya, Italy, and Thailand (Singha, 2012, Ortolani et al. 2015, Seedballs Kenya 2018). However, there is no report of what percentages of seeds germinate and establish as seedlings. Therefore, we still lack quantitative evaluation of the method in reducing seed predation and the effect on seed germination and seedling growth.

In direct seeding, two means for delivering seeds to degraded sites are by hand and by dropping from the air (aerial seeding). Aerial seeding is promising for large scale landscape restoration (Grossnickle and Ivetic 2017; Li et al. 2009). Aerial seeding can be performed with manned or unmanned aircraft and has been reported in many countries (e.g. Fortes 2006, Raffaella et al. 2015; Xiao et al. 2015). Manned aircraft can carry more weight and fly longer distances, but larger aircraft tend to fly at higher altitudes and speeds. On the other hand, an unmanned aerial vehicle (UAV) can fly much closer to the ground. Moreover, UAVs have lower operation costs and lower space requirements for taking off and landing. For forest restoration, aerial seeding by UAVs is a new and promising technique that requires technical development of specific seed delivery systems. Delivery systems need to be designed to carry and release seeds according to specific restoration plans. In addition, we need quantitative studies of seedling establishment after seeds reach the ground.

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6. Objectives

6.1. To compare seed removal percentages among seed enclosing treatments (Related to research question 1).

6.2. To determine coating substances that are able to increase seed germination percentage and rate (Related to research question 2).

6.3. To determine the relationship of seed and seedling traits of tree species and species' ability to establish in degraded area (Related to research question 3).

6.4. To calculate and compare performance index of tree species suitable for direct seeding (Related to research question 3)

6.5. To compare survival and growth of tree species in different treatments of direct seeding (Related to research question 1, 2, and 3).

6.6. To compare seed germination and seedling survival and growth between two studied areas and determine site factors that affect seedling establishment (Related to research question 4)

6.7. To develop a seed dispenser system to deliver seeds to a degraded area using a UAV (Related to research question 5)

6.8. To determine germination percentage of seeds, delivered by the UAV, and determine seedling survival and growth in degraded areas (Related to research question 5).

7. Methodology

7.1 Study sites and measurement of site conditions (Objective 6.6)

Field experiments will be conducted in two abandoned agricultural sites under the authority of Nong Hoi Royal Project (hereafter Mon Cham and Mon Long degraded area), Mae Rim District in Chiang Mai, Thailand. Experimental plots will be established at about 1000 - 1,500 m above sea level. At each degraded area, the experiments will cover an area of 4800 m^2. The biotic and abiotic conditions of the sites will be measured - natural seedling density and ground herbaceous plants, humidity, temperature and precipitation. In addition, landscape factors of slope and aspect will be recorded.

In addition to the field experiments, parallel seed germination tests will be conducted at a research nursery, 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).

7.2 Study species

Five native tree species from Northern Thailand will be used for the experiments. All of the species are present at high altitude (about 900-1,500 m above sea level). The species will be Hovenia dulcis Thunb. (Rhamnaceae), *Choerospondias axillaris* Roxb. (Anacardiaceae), *Prunus cerasoides* D. Don (Rosaceae), *Alangium kurzii* Craib (Alangiaceae), *Sarcosperma arboreum* Hook. (Sapotaceae). The seed size of the five tree species spans from 24 mg to 260 mg and the species have been reported to be suitable for the conventional tree planting method for restoring degraded areas of Northern Thailand (Elliott et al. 2003). In addition to their suitability for forest restoration, the tree species have been selected based on their reproductive schedules. Mother trees bear fruit before and during the rainy season, which is postulated to make them suitable for direct seeding (Tunjai and Elliott 2012). The seed removal percentage of four of the species has been studied in a previous TRF funded research MRG 5980177).

For each species, about 3,000 seeds will be collected from at least five mother trees. After seed collection, the seeds of all five species will be cleaned, air-dried, and stored at room temperature until the seeds are used. The collected seeds will be separated into three seed lots - (1) seeds to be used for seed trait measurement, (2) seeds to be sown in field and nursery experiments, and (3) seeds to be delivered by a UAV.

7.3 Experimental design and data collection (Figure 2)

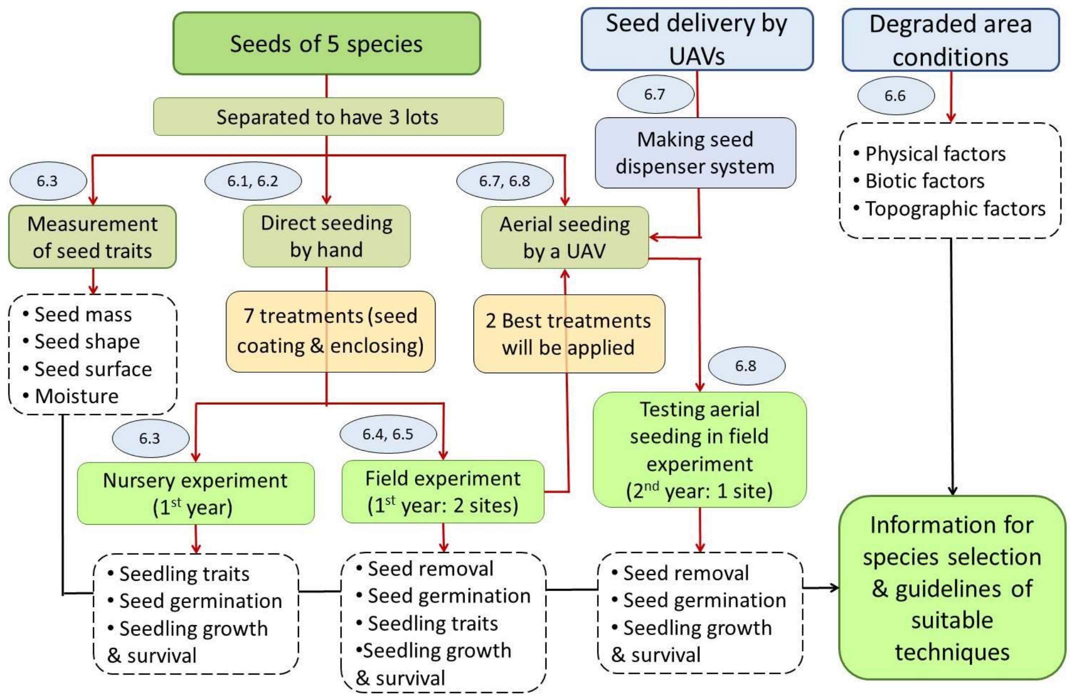

Figure 2 Experimental scheme of this proposed study. The numbers in ovals reference research objectives from section 6. The boxes with dashed-line border represent data to be collected.

7.3.1 Seed and seedling trait measurement (Objective 6.3)

For the first seed lot, 20 seeds of each tree species will be randomly sampled. Seed characteristics will be measured, including seed mass (mg), seed shape (width and 2 lengths), seed coat thickness (mm), seed surface structure and moisture content (%). The seeds will be oven-dried for dry mass and moisture measurement; therefore these seeds will not be viable for sowing. The seed coat will be separated for thickness measurement. The seed surface structure will be studied under a microscope. The length and width of the seeds will be measured using digital vernier calipers. In addition, the seed storage behavior will be classified as orthodox, recalcitrant or intermediate according to Hong and Ellis (1996) and Schmidt (2007).

For measuring seedling traits, twenty seedlings from the nursery experiment (described in 7.3.2) will be harvested 4 months after germination. Seedling traits will be measured, including number of leaves, specific leaf area, root collar diameter, height, and dry mass. Seed and seedling traits will be used to study relationships with seedling growth and establishment success in direct seeding.

7.3.2 Seed treatments in two degraded areas and a tree nursery (Objective 6.1, 6.2)

For the second seed lot, the seeds of each species will be pre-treated as per the recommendations in previous studies, for example, removing arils or scarifying seeds (e.g. cutting seed coat) (e.g. FORRU, 2006). The seeds will be surface sterilized using sodium hypochlorite solution.

In this study, the two approaches to address the seed removal/predation problem are (1) to enclose seeds with protective materials and (2) to promote seed germination and early seedling growth by coating seeds with beneficial substances. The first approach, which will be referred to as seed enclosing, is intended to add materials to disguise the seed’s smell and to make the seeds less attractive to animal removers. In this proposed study, soil particles will be used as the enclosing material. The second approach will be referred to as seed coating. The seeds will be coated with various materials under study, which have been reported to be beneficial for seed germination and early seedling growth. The coating materials will include biochar, super absorbent polymers (SAP), and actinobacteria. The two approaches will be crossed over, and in total of seven treatments will be applied (Table 1).

Table 1 Seven treatments to reduce seed removal (enclosing) and to promote seed germination and seedling growth (coating)

Promoting germination and growth / Enclosing (protection)	Actinobacteria	SAP	Biochar	No coating
Soil	T1	T2	T3	T4
No enclosing	T5	T6	NA*	T7 (Control)

*Not applicable because biochar cannot be used on its own. In this proposed study, the biochar will be mixed with soil.

The control treatment will consist of seeds without coating or enclosing. The soil used for enclosing the seeds will be sterilized by autoclaving. For the actinobacteria treatment, the actinobacteria to be used in this proposed study are *Streptomyces thermocarboxydus* isolate S3 (Lasudee et al. 2018). The isolates of *S. thermocarboxydus* will be supplied by Dr. Wasu Pathom-Aree, Department of Biology, Chiang Mai University. The seeds will be mixed with the *S. thermocarboxydus* in a rotary shaker before enclosing in soil.

For the SAP treatment, the active constituent will be acrylamide and potassium acrylate copolymer. The SAP will be mixed with clay and talcum powder. The mixture ratio will be 6:3:1 of clay, talcum powder and SAP (Su et al. 2017). The seeds will be coated with the SAP mixture in a rotary drum Ethylene cellulose dissolved in alcohol will be used as an adhesive solution.

For the biochar treatment, biochar from longan wood will be mixed with the enclosing soil and applied to the seeds. The biochar cannot be used alone due to its high basicity (pH 12-13) (Shafer, pers. comm.).

After seed coating and enclosing, the seeds will be divided to two groups. The first group of seeds will be sown by hand directly in two degraded areas – Mon Cham and Mon Long. A three-rai plot (4800 m^2) will be established at each degraded area. There will be three replicates of 20 seeds per treatment. For each replicate, bamboo tubes (about 10 cm long and 5-10 cm diameter) will be buried at five cm deep into the soil and 1.5 m apart from one another. In each tube, one seed will be pressed into the soil so that the surface of the seed is level with the soil surface. The number of seeds that are removed and the number of germinated seeds will be recorded. Germination will be determined by radicle emergence. Monitoring will end when no further germination occurs in four consecutive weeks.

To collect data on the effects of coating and enclosing materials on seed germination and seedling growth in a control environment, the second seed group will be sown in a tree nursery in parallel to the field experiments. One hundred seeds from each of the seven treatments will be sown in gardening polybags filed with forest soils. The polybags with seeds will be placed on the nursery ground, surfaced with plastic sheets. The experimental area will receive 30% sunlight. Seed germination will be determined by radicle emergence, as in the field.

7.3.3. Seedling performance in the degraded areas (Objective 6.4, 6.5)

During the first year of field experiments, weeds will be removed and the experimental seedlings will be given fertilizer every three months. The number of surviving seedlings will be recorded. To determine the performance of seedlings from the direct seeding method, the size of the seedlings – root collar diameter (mm) and height (cm) - will be measured when the fertilizer is applied. Moreover, relative growth rate (% per year) will be calculated according to Forest Restoration Research Unit (2008). The effects of seed coating and enclosing on seed germination and seedling growth will be compared among treatments and among species.

7.3.4 Aerial seeding by a UAV (also known as drone) (Objective 6.7, 6.8)

In the first year, a seed dispensing system will be designed and built. The seed dispenser will consist of a seed container and a mechanism to release seeds from an aperture. The dispensing system will be fitted to a DJI Phantom 4 drone (Figure 3) and the system will be tested at the Department of Biology before actual aerial seeding in the field.

In addition during the first year, seed delivery darts (Figure 4) will be designed and constructed. The darts will be made of biodegradable paper. The purpose of the darts is to ensure that seeds are secured on the ground upon arrival. The design and prototype will be tested at the Department of Biology before actual aerial seeding in the field.

Figure 3 A drone owned by Forest Restoration Research Unit to be used for testing the prototype seed disperser system

In the second year, the two best seed coating and enclosing treatments will be selected for aerial seeding. The enclosed seeds will be put in delivery darts, as designed in the first year. Each dart will contain one seed. The 200 aerial darts will be delivered to the site by UAV. The delivery height will be 40 m from the ground of the degraded site. After delivery, all seeds will be visually located and flagged, and seed germination and seedling survival and growth will be recorded.

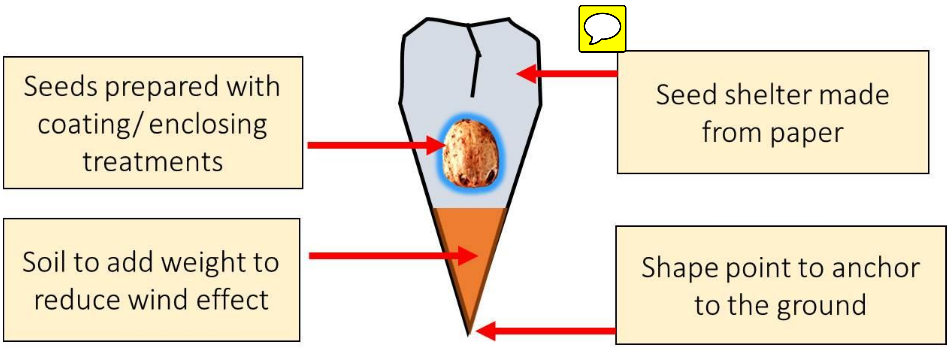

Figure 4 Conceptual design for an aerial seed dart

7.6 Data analysis (Objective 6.1-6.8)

Data analysis will be performed using the R programming language (R Development Core Team). The variables of interest and statistical analysis methods corresponding to each research objective are shown in the variable table in section 8.1 of scope of research.

7.6.1 Effects of seed treatments on seed removal, germination, and seedling growth (Objective 6.1, 6.2, 6.5)

The percent removal and percent germination of seeds will be calculated for each treatment. A generalized linear model (GLM) with binomial error will be used to test the effect of treatments on seed removal and seed germination. In addition, the percent germination of seeds, mortality rate, survival of seedlings, and relative growth rate will be compared between direct seeding and nursery production.

The relative growth rate of the seedlings will be calculated and compared among treatments. Analysis of variance (ANOVA) will be used to test whether seedling performance differs among these treatments. In addition, seedling survival will be analyzed using survival analysis to determine if the survival differs among treatments.

7.6.2 Relationship among traits and seedling performance (Objective 6.3)

Regression analysis will be used to explore the relationships among seed and seedling traits, and germination and seedling performance. The seed traits include germination percentage and rate, seed mass (mg), seed shape (width and 2 lengths), seed coat thickness (mm), seed surface structure and moisture content (%). The seedling traits include specific leaf area, root collar diameter, height, and dry mass. The seedling performances of interest are survival and growth.

7.6.3. Evaluate species suitability for direct seeding (objective 6.4)

To determine whether species are suitable for direct seeding, a species performance index (SI) will be calculated using data from the field experiment (Naruangsri 2017). The SI is based on a combination of seed and seedling criteria including seed removal percentage, seed germination, seedling survival, and seedling growth rate. The species will be classified according to their SI value as excellent, good, marginal, or poor species for direct seeding.

7.6.4. Comparison of seed germination, seedling survival and seedling growth between degraded areas (Objective 6.6)

The biotic and abiotic conditions of the degraded areas may influence germination and seedling performance. The percentages of seed germination, seedling survival, and seedling relative growth rate will be compared among the two studied sites using Student's

T-test. Also, the physical, biotic and topographic factors will be compared among the two sites.

7.6.4 Success of aerial seeding (objective 6.8)

Seed germination percentage, seedling survival percentage and seedling relative growth rate will be calculated after the first rainy season. A GLM with binomial error will be used to compare among the species, treatments and degraded areas. Moreover, the degradation time of the biodegradable aerial-darts will be recorded in the field.

8. Scope of research

8.1 Variables of interest

Variables are ordered by the research questions and the objectives

Objectives*	Independent variable	Dependent variable	Data analysis
6.1	Different enclosing treatment	Seed removal probability	Generalized linear models
6.2	Different treatment of seed coating	Seed germination	Generalized linear models
6.3	Seed size, seed coat thickness	Seed germination and seedling growth	Regression
6.4	Different species	Performance index based on minimum standard approach	Computing score and compare among species
6.5	Different seed enclosing and coating treatments	Seedling survival and growth rate	Survival analysis and ANOVA
6.6	Two studied sites	Percent seed germination, seedling survival and seedling growth rate	Student's T-test
6.8	Aerial seeding treatments	Seed germination	Generalized linear models
6.8	Aerial seeding treatments	Seedling survival and growth rate	Survival analysis and ANOVA

*There is no variable for data analysis for Objective 6.7 of developing delivery systems

8.2 Seed source

Five native forest tree species of Northern Thailand will provide seeds for the experiments. At least 3,000 seeds of each species will be collected from tree populations of Doi Suthep-Pui National Park.

8.3 Measurement of seed germination and seedling survival and growth

Seed germination is a process involving absorption of water by seeds and emergence of plant parts from quiescent seed stage. Seed germination can be measured by > 2 mm of radicle emergence.

For seedlings, survival percent can be expressed as a proportion of seedlings present at the time of measurement and seedlings at the beginning of the experiment. Seedling growth can be quantified by changes in size of plant parts. For growth measurement, the size of the seedlings – root collar diameter (mm), height (cm) - will be measured and relative growth rate (% per year) will be calculated (Forest Restoration Research Unit, 2008).

8.4 Components of the proposed study

1. ***Direct seeding treatment preparation***

1.1. *Streptomycete thermocarboxydus* treatment

Actinobacteria have been reported to be beneficial to plants. In this study, the *S. thermocarboxydus* isolate S3 (Lasudee et al. 2018) will be used because the evidence in growth promoting benefits to seedlings of rice. However, benefits to forest tree species have not been studied. The preparation of *S. thermocarboxydus* will be done in a microbiology laboratory at the Department of Biology, Chiang Mai University. I will collaborate with Dr. Wasu Pathom-Aree in supplying *S. thermocarboxydus* isolate S3 and microbiology technical support.

1.2. Super absorbent polymer (SAP) treatment

The active constituent of SAP will be acrylamide and potassium acrylate copolymer. The SAP mixture will be prepared as reported in a previous literature by Su et al. 2017. The coating process will require filling materials, including clay and talcum powder, and adhesive solution of ethylene cellulose in alcohol.

1.3. Biochar treatment

Biochar will be supplied by Warm Heart Foundation in Chiang Mai, Thailand. The biochar to be used in this study is made by burning longan wood. The chunks of biochar will be crushed and ground to small particle size and mixed with soil before seed enclosing.

2. ***Direct seeding in the field*** – to test the effectiveness of treatments on reducing seed removal and promoting germination and growth of seedlings

The seeds will be prepared according to the experimental treatments (in 7.3.2). At the beginning weeds in two sites will be cleared. The experimental seeds will be placed in two studied sites - Mon Cham and Mon Long. After sowing seeds, weed in the experimental sites will be removed by cutting every three months. Then the seed removal, seed germination, seedling survival and growth will be compared among treatments and among sites.

3. ***Testing in a tree nursery –*** to test the effect of treatment materials on seeds and seedlings

The seeds, prepared with the same experimental treatments for direct seeding, will be sown in a tree nursery. The seeds will be watered according to the common nursery care. Seed germination, seedling survival and growth will be compared among treatments. In addition, these lots of seeds prepared in the same fashions of the experimental treatments will allow closer examination of changes to the experimental materials.

4. ***Seed and seedling trait measurement***

One of the seed lots will be kept and used in seed trait measurement. The seeds will be randomly sampled from the seeds collected at the same time.

Seedlings will be grown in the tree nursery in Doi Suthep Pui National Park. The seedlings will be water every day until measurements. At the time of seeding trait measurement, the seedlings will be four months old counting from the day of germination is observed.

5. ***Making of seed dispenser system using with a UAV – aerial seeding***

A consumer UAV will be used in this study. The consumer UAV means the UAV that is available in the market and can be bought off the shelf. In this study, a DJI Phantom 4 drone will be used. One benefit of the consumer UAVs is that the UAVs are available for general public. Results of this proposed study will be easily transfered to general public.

A seed dispenser system will be designed and built to fit with the DJI Phantom 4. The system will be consisted of a seed container and a mechanism to release seeds from an aperture.

6. Aerial testing in the field

After the see dispenser system are done, the field test of aerial seeding using the UAV will be carried out in one of the field sites. The seeds will be prepared with the two treatments with the best seed germination result and each seeds will be packaged in an aerial dart. Then the seeds will be delivered to the site by a DJI drone. Seed germination and seedling survival and growth will be recorded.

9. Equipment needed for the project

	Equipment	
	Already have	**Equipment that will be made or borrow**
1	Tree nursery	Seed enclosing drum
2	Computer for data entry	Seed container to carry seeds on the drone
3	DJI Phantom 4 drone	Seed release mechanism working with the drone
4	Digital Vernier calipers	Data loggers temperature and humidity sensors
5	Stereoscope	GPS

10. Project duration 2 years (24 months)

11. Schedule for the entire project and expected outputs

		Month of operation																							
Objective	Activities	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
Preparation	Preparing materials and sites																								
Maintenance	Site maintenance																								
Preparation and 6.3	Seed collection and trait study																								
6.6	Site conditions measurement (biotic and abiotic factors)																								
6.1 – 6.6	Experiments in the field and data collection																								
6.1 -6.3	Experiment in the tree nursery and data collection																								
6.3	Seedling trait measurement (nursery seedlings)																								
6.7, 6.8	Designing seed dispenser and aerial darts																								
6.7, 6.8	Selecting treatments and species & preparing seeds																								
6.7, 6.8	Aerial seeding by UAV and data collection																								
Finish up	Experimental site clean-up																								
6.1 – 6.8	Data analysis																								
Knowledge distribution	Report preparation & submission																								
Knowledge distribution	Presentation at a conference																								
Knowledge distribution	Preparing & submitting manuscript																								

11.2 Publication output

I will prepare a manuscript, provisionally entitled “Seed treatments to enhance success in direct seeding methods for tropical forest restoration in Northern Thailand”. I will submit the manuscript to Restoration Ecology (impact factor (2015) 2.54)

11.3 Expected outputs by year

Objectives	Expected Outputs	1st year	2nd year
6.1	New knowledge on reducing seed removal and predation in degraded area of Northern Thailand	/	
6.2	New knowledge on increase seed germination success in degraded area of Northern Thailand	/	
6.3, 6.5	A better understanding of the relationship among seed and seedling traits and seedling establishment success	/	/
6.3	Useful data of seed and seedling traits of native tree species of Northern Thailand	/	
6.1, 6.2, 6.4, 6.5	Information for decision making whether to use direct seeding for forest restoration and what species are suitable	/	/
6.6	Information for decision making about what site conditions are suitable to use direct seeding	/	/
6.7	Seed dispenser prototype to be use with a UAV	/	
6.7, 6.8	Protocol for aerial seeding for forest restoration to be use as a guideline for further development of techniques		/
6.1 - 6.8	Teaching lessons in plant ecology about techniques for direct seeding and aerial seeding	/	/
6.1 - 6.8	Published article in an international journal and presentation at conferences		/

12. Budget details

The amount is in Thai Baht.

Budget detail	1st year	2nd year	Total
1. Honorarium			
- Pimonrat Tiansawat (13,000 baht per month)	156,000	156,000	312,000
2. Wage for research assistant			0
- Research assistant goes in the forests, and degraded areas with the PI and helps with experiments (4,000 baht/month: 20 months)	48,000	32,000	80,000
3. Materials			
- Materials for polymer coating experiment	10,000		10,000
- Materials for actinobacteria culture and coating experiment	15,000	5,000	20,000
- Seed storage box	1,000	1,000	2,000
- Planting materials (tray, soil, compost, poly bags)	4,000	2,500	6,500
- Tree monitoring tools (measuring tapes, poles)	300	200	500
- Stationery for data collection	500	2,000	2,500
- Materials to make seed container and dispenser system		5,000	5,000
4. Expenses			
- Transportation to field sites (1st year) (15 trips)*	45,000		45,000
- Transportation to field sites (2nd year) (24 trips)*		72,000	72,000
- Food and drinks for field work 1st year (200 baht per field trip, 15 field trips per year)*	3,000		3,000
- Food and drinks for field work 2nd year (200 baht per field trip, 24 field trips per year)*		4,800	4,800
- Labor cost for making seed coating drum (hiring)	3,000		3,000
- Site maintenance cost: labor cost for weeding (hiring)	5,000	5,000	10,000
- Labor cost for drone modification (hiring)	4,000	4,000	8,000
- Photocopy (photocopying of data sheets, articles, books)	200	500	700
- Travel support to TRF events	5,000	5,000	10,000
- Registration fee for presenting at a conference		5,000	5,000
Total (Baht)	300,000	300,000	600,000

* Two field sites are about 40 km from the university. Access to the sites = rental 4WD and/or motorcycles. Food will be prepared for full-day work in the fields.

13. Expected benefits

13.1 Expected benefits to academic community

This proposed study will advance forest restoration science. The finding in this work will be used in teaching plant ecology and restoration ecology.

Benefits	Users
1.New knowledge on the effects of seed enhancement techniques in minimizing seed predation and promoting seed germination and seedling growth in degraded areas. (research objective 6.1, 6.2)	Researchers, students, forest restoration practitioners, general public wanting to restore land
2.Data on how seed traits relate to seedling establishment in degraded areas and performance index for different species. The data will help to design better restoration plans. (research objective 6.3, 6.4)	Researchers, students, forest restoration practitioners
3. Data on seedling growth and survival in degraded areas can be used in modeling seedling regeneration in degraded areas. (research objective 6.5, 6.6)	Researchers, students, forest restoration practitioners
4.This study merges forest restoration science with current UAV technology. This study opens up new ways to use UAV technology. Also the study provides ecologists a new approach to use UAVs for ecological studies. (research objective 6.1 - 6.8)	UAV developers, researchers, students, forest restoration practitioners

13.2 Expected benefits to society

The knowledge gained from this study will be used in restoring forests. The field site is in degraded areas of the Nong Hoi Royal Project.

Benefits	Users
1.This proposed study will provide guidance for planning towards Thailand's target of 40% forest cover.	Policy makers
2.This proposed study will provide information on species selection and site management for forest restoration by direct seeding.	Forest restoration practitioners, private sectors and general public wanting to restore lands
3.The findings from this study will help with deciding which methods are suitable for restoring forests in certain areas. The most relevant information is how to work with seeds and treatments for better seedling growth.	Policy makers, forest restoration practitioners, private sectors and general public wanting to restore lands
4.The proposed study will emphasize the importance of restoring forests in Thailand and worldwide.	Policy makers, private sectors, school and college students, and villagers

14. Connections with other experts within and outside Thailand

14.1 Connections in Thailand

1. Dr Stephen Elliott (mentor; Department of Biology, Chiang Mai University), an expert in tropical forest restoration and co-founder of the Forest Restoration Research Unit of Chiang Mai University.
2. Prof. Saisamorn Lumyong (Department of Biology, Chiang Mai University), an expert on microbiology and utilization of endophytic microbes as plant growth promoters.
3. Assist Prof. Dr Wasu Pathom-Aree (Department of Biology, Chiang Mai University), an expert on actinobacteria and utilization of actinobacteria.
4. Dr Dia Panitnard Shannon (Department of Biology, Chiang Mai University), an expert in direct seeding method for restoration.

5. Assist Prof. Dr Annop Ruangwiset (Department of Mechanical Engineering, King Mongkut's University of Technology Thonburi), an expert on UAV Design, Control and Navigation

14.2 Connections outside Thailand

1. Prof. Dr James Dalling (University of Illinois), an expert in seed ecology, plant-animal interaction.
2. Simone Pedrini (King Parks and Curtin University), his research focus is on seed enablement technology (seed coating and petting).
3. Assist Prof. Dr Noelle Beckman (Utah State University), an expert on ecological data analyses and modeling. Her work is also focus on seed ecology

15. Will this proposal or related proposal be submitted within the next six months or has it been submitted to other funding agency/source?

☑ No

☐ Yes, please give the name of the funding agency, name of the project and status of submission.

16. The projects that the principal investigator is currently carrying out

16.1. Project title: Seed and seedling predation of five native tree species of Northern Thailand: an implication to direct seeding for forest restoration

Project duration: 2 years from May 2016 to May 2018 (1^{st} extension to November 2018)

Research funding institute: The Thailand Research Fund

Budget: 600,000 Baht

Role of principal investigator in this project ☑ principal investigator

☐ other (please specify)

Weekly hours spent on this project: 18 hours/week

16.2. Project title: Increase capacity of tropical forest restoration through modeling tree species distribution

Project duration: 2 years from January 2016 to December 2018

Research funding institute: Research Fund for DPST Graduate with First Placement

Budget 400,000 baht

Role of principal investigator in this project ☑ principal investigator

☐ other (please specify)

Weekly hours spent on this project: 7 hours/week.

16.3. Project title: From a bare mountain to a regenerated forest: comparing landscape planting design for forest restoration in Nan province

Project duration: 2 years from September 2017 to August 2019

Research funding institute: National Science and Technology Development Agency

Budget: 4,109,000 baht

Role of principal investigator in this project ☐ principal investigator

☑ other:

Role of principal investigator in this project:

Collaborator in the project responsible for phenology study of trees in Nan

Weekly hours spent on this project: 7 hours/week