CHAPTER 3

METHODOLOGY

3.1 Study site

This research was conducted in Doi Suthep-Pui National Park, (18°51′N latitude and 98°54′E longitude), Chiang Mai, Northern Thailand. The park supports three forest types: deciduous dipterocarp-oak forest from sea level to *c.* 800 m., mixed forest (evergreen + deciduous) from *c.* 800 to 1,200 m. and hill evergreen forest above 1,200 to the summit at 1,685 m. (Maxwell and Elliott, 2001). The area has a monsoonal climate with pronounced dry and wet seasons. The wet season lasts from May to October and the dry season from November to April. The dry season is subdivided into the cool-dry season (November to January) and the hot-dry season (February to April; Fig. 4).

Ficus spp. trees were propagated in the research nursery of the Forest Restoration Research Unit (FORRU-CMU) at 1,050 m elevation, in the south of the park, north-west of Chiang Mai City, Northern Thailand (18°51′N latitude and 98°54′E longitude). Experiment plots were established in a degraded watershed, in the north of the park (18°52′N latitude and 98°51′E longitude) at 1,150 m above sea level. The plots had been cleared of forest approximately 30 years ago, to provide land for cultivation of cabbages, corn, potatoes etc. Before tree planting, the plots were dominated by herbaceous weeds such as *Pteridium aquilinum* (L.) Kuhn, *Bidens pilosa* L. var *minor* (Bl.) Sherf and grasses e.g. *Phragmites vallatoria* (Pluk. ex L.)

Veldk, *Imperata cylindrical* (L.) P. Beauv var. major (Nees) C. E. Hubb. ex Hubb and Vaugh (pers. obs).

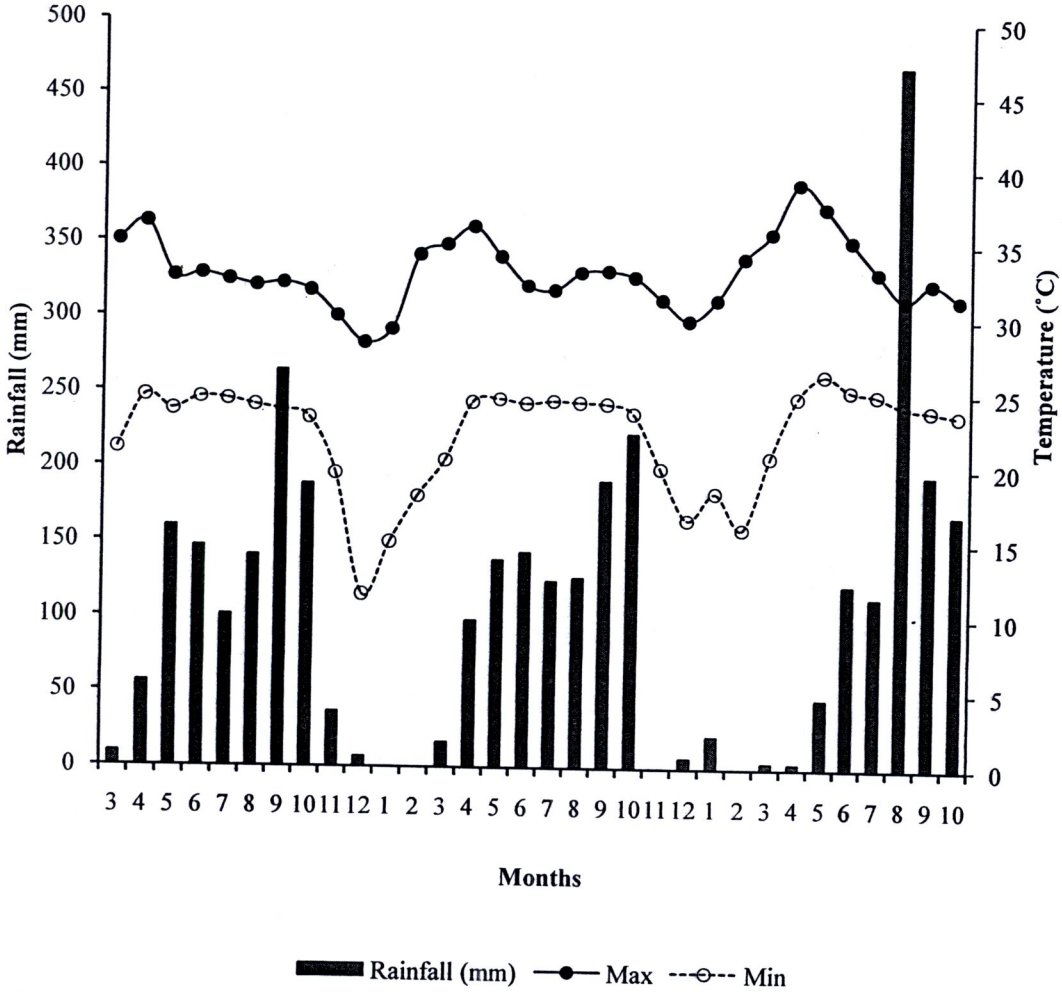

Figure 4 Average monthly rainfall (mm), maximum and minimum temperature (°C) at the Northern Meteorological Center, about 3 km. from the National Park (from March 2008 - October 2010).

3.2 Study species

3.2.1 ***Ficus auriculata*** Lour. (subgenus *Sycomorus*, section *Sycomorus*, subsection *Neomorphe*). *Ficus auriculata* is a medium-sized tree, up to 20 m tall,

becoming shortly buttressed. Young leaves are red, becoming green when mature. Figs grow on spurs that extend from the trunk, main branches (cauliflorous form) and sometimes from the crown roots (stoloniflorous form). The fig is reddish or purple at maturity. The female figs and young leaves are edible. This species grows in mixed deciduous and evergreen forests, often near streams, on various substrates (Berg *et al.*, 2011). Figs production commences from the 5th year after planting (FORRU, unpubl. data, 2004).

3.2.2 *Ficus oligodon* Miquel (subgenus *Sycomorus*, section *Sycomorus*, subsection *Neomorphe*). The morphology of *F. oligodon* and *F. auriculata* is very similar. While the two species are confused in the latest taxonomic revision (Berg *et al.*, 2011), they are readily recognizable in the field. In our research nursery (FORRU-CMU), external characters of leaf shape/size (*F. auriculata*: broad leaves and *F. oligodon*: narrower often toothed leaves), and the color of petiole of young seedlings or saplings (*F. auriculata*: greenish and *F. oligodon*: reddish) are used to separate *F. auriculata* from *F. oligodon.* *Ficus oligodon* is a spreading tree, which grows in all forest types in Doi Suthep-Pui National Park (under the name of *F. fistulosa* Reinw. ex. Bl. var *fistulosa* in Maxwell and Elliott, 2001). Figs are borne singly or in small clusters from tubercles on the trunk and a larger branch. The female figs and young leaves are edible, and it is also an excellent host tree for Lac insects (*Laccifer lacca* Kerr., per. obs.). They produced figs from the 6th year after planting and are very attractive to seed-dispersing birds (FORRU, 2006).

3.2.3 *Ficus variegata* Blume (subgenus *Sycomorus*, section *Sycomorus*, subsection *Neomorphe*). *Ficus variegata* is a pioneer, large-sized tree (up to 40 m tall) with prominent buttresses. The bark is smooth, pale pinkish brown (the tree is

sometimes named the red stem-fig). Leaves are thin, heart-shaped and have a toothed edge. The figs grow in dense clusters on the trunk and main branches. The fig is pink to red (or sometimes green) at maturity. It is mostly found in evergreen forest throughout the country, often near streams (Berg *et al.*, 2011). However, some individuals are found in relatively open forest as isolated trees (per. obs.).

3.2.4 ***Ficus hispida*** L.f. (subgenus *Sycomorus*, section *Sycocarpus*). *Ficus hispida* is a small or moderate-sized tree. Leaves are very rough and generally opposite. Through Thailand, the rough leaves are used to clean off mucilage from eels before cooking. Figs are axillary, cauliflorous and flagelliflorous. Figs are yellowish when ripe. This species grows in all forest types throughout the country, but it is most common in secondary growth, in drier climates, at low altitudes. All parts of this plant can be used in tradition medicine for the treatment of various ailments, for example, their stems have been utilized for the treatment of human breast cancer (Pratumvinit *et al.*, 2009). In forest restoration projects, this species has been ranked as an excellent framework species (Elliott *et al.*, 2003). Planted saplings produced figs within 3 years after planting and showed excellent weed suppressing capabilities and fire resilience (FORRU, 2006).

3.2.5 ***Ficus semicordata*** Buch.-Ham. ex Sm. (subgenus *Sycomorus*, section *Hemicardia*). *Ficus semicordata* is a tree with wide-spreading branches, brown hairs on leafy twigs, leaf and syconium. Leaves are alternate, prominently asymmetric especially at the base, and the mature leaves are rough and scurfy. This species is cauliflorous. The leafless fig-bearing branches develop at the base of the trunk and often become stolon-like, trailing across the forest floor. At some distance from the trunk such branches may start to grow upwards and become leafy, establishing

satellite trees. Externally the figs are red-brown at maturity. This species grows in all forest types, but mainly in disturbed areas and secondary growth forest. The female figs are edible and sweet. In forest restoration projects, this species is easy to propagate from seeds (Kuarak *et al.*, 2000). Planted saplings grow very rapidly and figs are produced prolifically from the 3rd year after planting (FORRU, 2006).

3.2.6 *Ficus fulva* Reinw. ex Blume (subgenus *Ficus*, section *Eriosycea*, subsection *Eriosycea*). *Ficus fulva* is a small tree, growing in the understory of mixed evergreen-deciduous forest. Whereas, in Malaysia, *F. fulva* is very common on small ridges, sifting cultivation and large landslide gaps in the primary forest (Harrison *et al.*, 2000). It forms almost pure stand with wide spreading branches and dense foliage. Twigs, leaves and figs have short white (silky hairs) bristles. The figs are axillary on the twigs, in pairs below the leaves on previous season's growth. The interior is scarlet and yellow to orange at maturity. The main seed dispersers of *F. fulva* are bulbuls (e.g. *Pycnonotus goiavier* Scopoli) and fruit bats (Harrison *et al.*, 2000).

3.2.7 *Ficus triloba* Buch.-Ham. ex Voigt (subgenus *Ficus*, section *Eriosycea*, subsection *Eriosycea*). *Ficus triloba* is a tree with stiff brown hairs on various parts. The leaves are borne in spiral and are often subpalmately 3-5-7-lobed. The figs are axillary, sessile, and may present lateral bracts. At maturity, it is yellow to red-brown. *Ficus triloba* was found only the forest understory of primary mixed evergreen-deciduous forest in the park, whilst Berg *et al.* (2011) reported that it can also be found in deciduous dipterocarp forest. In restoration plots, saplings produced figs within 5 years after planting (per. obs). More details of the seven selected dioecious *Ficus* species are presented in Table 2 and Appendix A.

Table 2 Overview of distribution, habitat and abundance of the seven selected dioecious *Ficus* species.

***Ficus* Species** [a]	**Distribution Range** [b] (Country)	**Habitat** [c]	**Elevation** [d] (m)	**Abundance Rank** [e]
F. auriculata	Bhutan, Cambodia, China, India, Laos, Myanmar, Nepal, Pakistan, Thailand, Vietnam	streams in dof, egf, eg/pine	891-1,319	rare
F. fulva	Brunei, China, India, Indonesia, Malaysia, Myanmar, Thailand, Vietnam	da, sg in egf	923-1,100	rare
F. hispida	Australia, Bhutan, Cambodia, China, India, Indonesia, Laos, Malaysia, Myanmar, Nepal, Sri Lanka, Thailand, Timor, Vietnam	da in bb/df, sg	326 -1,351	medium
F. oligodon	Bhutan, China, India, Malaysia, Myanmar, Nepal, Thailand, Vietnam	da, open bb/df, mxf, egf, sg	605-1,336	medium
F. semicordata	Bhutan, China, India, Malaysia, Myanmar, Nepal, Thailand, Vietnam	sg, da in bb/df, egf, eg/pine	418-1,531	medium

Table 2 (continued)

Ficus Species [a]	Distribution Range [b] (Country)	Habitat [c]	Elevation [d] (m)	Abundance Rank [e]
F. triloba	Bangladesh, Bhutan, China, India, Indonesia, Laos, Malaysia, Myanmar, Nepal, Vietnam	da, sg in egf	1050-1300	rare
F. variegata	Australia, Cambodia, China, India, Indonesia, Japan, Laos, Malaysia, Myanmar, Philippines, Thailand, Vietnam	egf	899-1343	rare

[a] All selected *Ficus* species were dioecious; free-standing.

[b] Source: Flora of China (Zhekun and Gilert, 2003); Flora Malesiana (Berg and Corner, 2005); Flora of Thailand (Berg *et al.*, 2011).

[c] Source: Maxwell and Elliott (2001); dof = deciduous dipterocarp-oak forest, egf = primary evergreen forest, eg/pine = evergreen forest with pine, da = disturbed areas, sg = secondary growth, bb/df = degraded teak & bamboo + deciduous forest.

[d] Altitude range (m) of the parent/donor trees were found along the seed/cutting collection trails of Doi Suthep-Pui National Park.

[e] Species abundance in the park is ranked by Maxwell and Elliott (2001); *F. fulva* is a synonym of *F. hirta* Vahl var. *roxburghii* (Miq.) and *F. oligodon* is a synonym of *F. fistulosa* Reinw. ex Bl. var. *fistulosa.*

3.3 Methods

3.3.1 *Ficus* phenology. Two transect lines were established to study the phenology of these species, traversing every forest type found in the National Park (from 320 to 1,685 m. elevation). Trail A (yellow line) ran across the park from east to west, whilst trail B (red line) ran from north to south (Fig. 5).

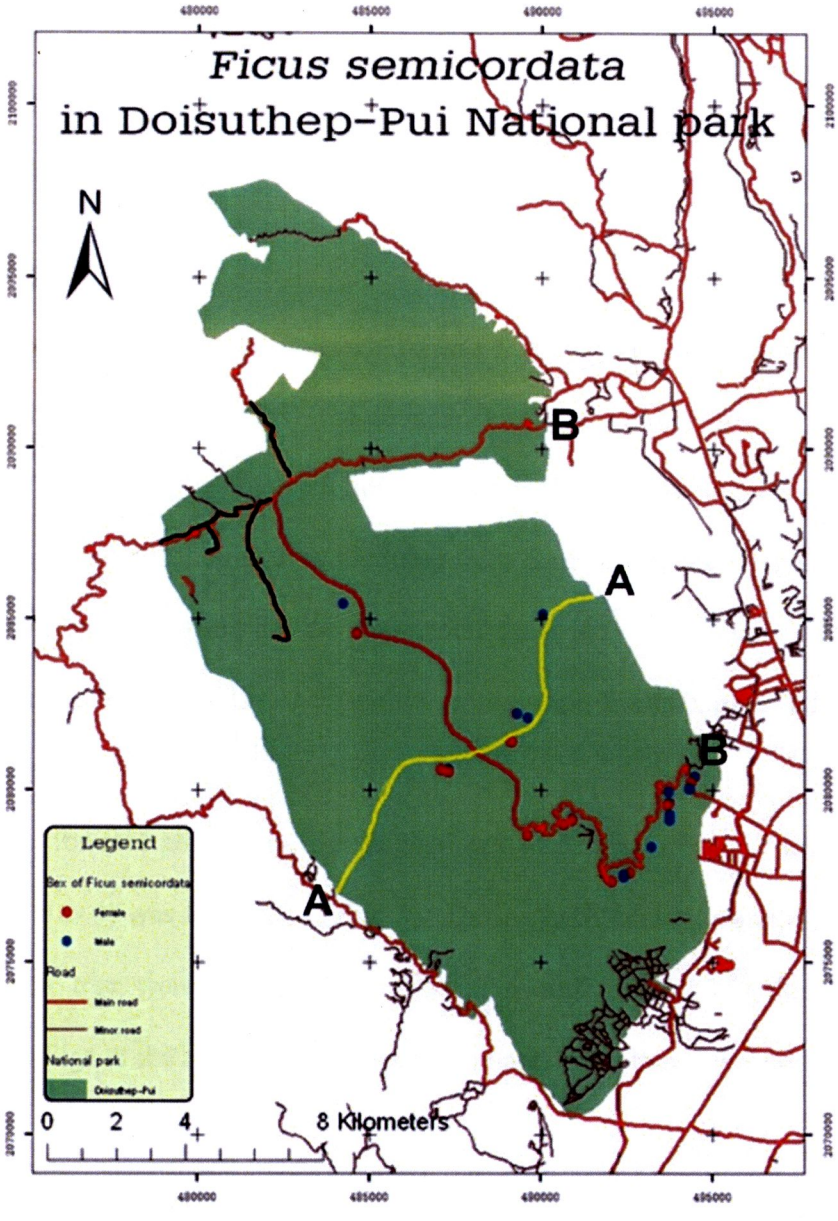

Figure 5 Map of the main part of Doi Suthep-Pui National Park and two phenology trails (A and B) along the park.

All mature individuals of the selected *Ficus* species (dbh >10 cm), within 20 m to the left and right of the transect lines, were selected for monitoring, tagged and their position recorded by GPS. Selected fig trees were observed monthly from March 2008 to February 2009. They were scanned with binoculars and scored for different pheno-phases of figs and leaves, using the crown density method (Koelmeyer, 1959). This method uses a linear scale of 0-4; with 4 representing the maximum intensity of figs or leaves. Values of 3, 2, and 1 represent three quarters, half and one quarter of the maximum intensity respectively. A value of 0.5 was used to indicate the presence of small amounts of figs and leaves below one-quarter of the maximum intensity.

Since the habits of the seven selected *Ficus* tree species were different (figs in leaf-axils, stem-figs and earth-figs), the abundance of figs was assessed in relation to the density of fig-bearing spurs or stolons on each tree. The scoring system for the developmental phases of the figs was modified from Galil and Eisikowich (1968) and Koelmeyer (1959) by splitting the developmental cycle into four pheno-phases (Fig. 6) and by using a linear scale of 0-5 (with 5 representing the maximum intensity of figs).

However, for leaf phenology, the original crown density method (using scores ranging from 0 to 4) was followed (Table 3). During each census, samples of 10-20 figs from each tree were collected for dissection and determining the stage of development. Figs at the receptive and ripening phases of both sexes were measured with a calipers and the number of seeds/wasps inside were also counted.

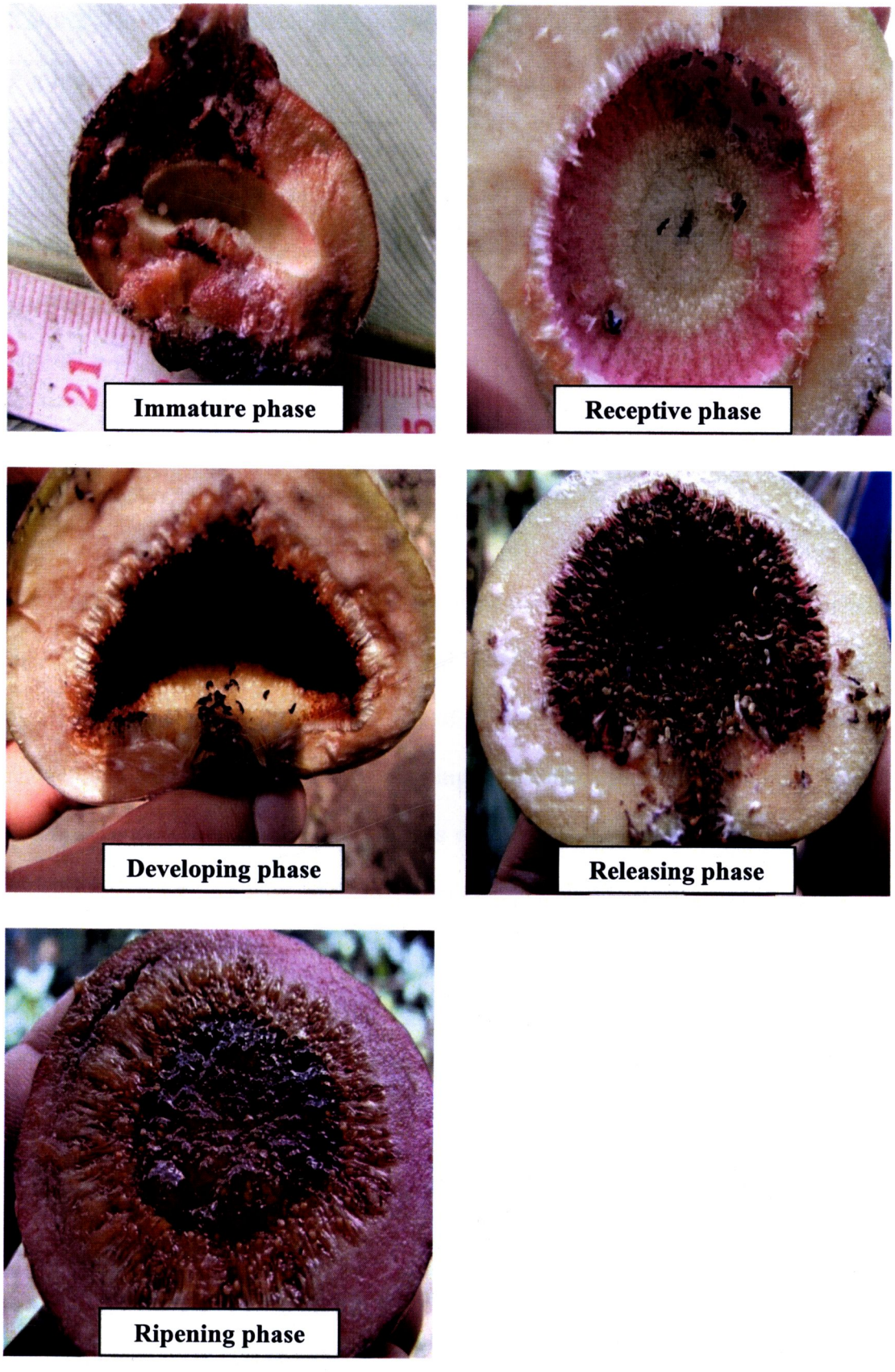

Figure 6 Stages of fig development of *Ficus oligodon*.

Table 3 The scoring system on figging and leafing phenology.

Fig Index [a]	**Leaf Index** [b]
NON-Phase = No figs on each tree	BA = Bare branches, leaf fall
IMP-Phase = Immature phase	YL = Young leaves
RCP-Phase = Receptive phase	ML = Mature leaves
DVP-Phase= Developing phase	SL = Senescence leaves
RPP/RLP-Phase = Ripening phase (female trees) / Releasing phase (male trees)	

[a] The sums of fig index have to be equal 5.

[b] The sums of leaf index have to be equal 4.

3.3.2 Fig wasp collection. The fig wasp community was studied on seven dioecious *Ficus* species in Doi Suthep-Pui National Park, northern Thailand, from March 2008 to February 2009. Male figs of all selected *Ficus* species were collected and cut into halves during the early releasing phase, when figs still had no exit holes (10 figs from each of five trees). The figs were stored at room temperature (ca. 25-30°C) in glass jars covered with fine nylon cloth, to allow the fig wasps to emerge. All emerging wasps were collected and preserved in 70% alcohol, and then dried in the laboratory of Centre de Biologie et de Gestion des Populations (CBGP), Montpellier, France by using hexamethyldisilazene (HMDS) method.

HMDS method

1. Fig wasp specimens were removed from 70% alcohol and soaked in 96% alcohol (in a microporous basket) twice (30 minutes per time).

2. They were then put into absolute alcohol (100%) twice (30 minutes per time). The purpose of these two baths was to totally dehydrate the insects.

3. They were soaked again in Hexamethyldisilazane (HMDS) twice (30 minutes per time).

4. Finally, specimens were put in an incandescent lamp. The drying time depended on the temperature: at 25°C = 8 hours, at 45°C = 4 hours and at 60°C (under a lamp) about 1 hour.

Later, the specimens were ready for mounting and photography. During the mounting process, the dried wasps were glued onto the side of a card (including ovipositor), wings were removed above and then the wasps were put flat under a microscope for detailed study. The specimens were photographed with digital camera (LEICA Z16 APOA) using the Cartograph program. Identifications were made using available keys (Wiebes, 1994) with the help of J. Y. Rasplus of the Centre de Biologie et de Gestion des Populations (CBGP), Montpellier, France, and by comparison with reference collections from China held by J.Y. Rasplus.

3.3.3 Seed/foundress collection. During each census of the phenology study (12 times from March 2008 to February 2009), samples of 10-20 figs from each tree along the two transect lines, traversing different habitats (Table 4) were used to investigate the stability of fig tree and fig-wasp populations. All seeds/wasps were counted in each fig at the ripening phase. To investigate the number of foundresses, figs were dissected in early DVP-phase, when foundressess are still intact and easy to find. Figs were cut into four pieces, and the heads of foundresses counted from each fig. Figs at the receptive and ripening phases of both sexes were measured with calipers.

Table 4 The three sampling sites were selected on basis of the distribution of fig trees and the degree of human disturbance.

Sampling sites	Site description
Primary forest	Fig trees are still in groups (>10 trees per sq. km). The area is generally well conserved with >90% of the area covered by forests.
Restoration plot	The Ban Mae Sa Mai plot, located at the north end of DSNP, approximately 2 km from primary forest. The surrounding area is mainly fragmented by crop fields. *Ficus* spp. were planted from 1999 to the present by FORRU, averaging about 2-10 fig trees per sq. km.
Highly disturbed	Outside the conservation areas, difficult to find the other fig trees within 1 sq. km (1 trees per sq. km). The areas were highly disturbed by people and infrastructure (e.g. campus, farm, urban).

3.3.4 *Ficus* propagation. All seeds and cuttings were collected from trees beside dirt tracks which run through natural or disturbed forest ecosystems of the park.

3.3.4.1 Propagation from seed. Mature, ripe figs of the six selected *Ficus* spp. were collected from 10 or more individual trees of each species. Fruits were removed directly from the plant, rather than harvested from the ground, principally to reduce the risk of diseases-infection (FORRU, 2006). Figs were opened and the seeds

scraped out with a spoon. The pulp was sieved through a mosquito net in water, so that viable seeds passed through the mosquito net and sank. Seeds were spread out on paper and left to dry for 1-2 days being sown into modular plastic trays, by placing them on the surface of the germination medium, uncovered. The effects of varying the composition of the germination medium, and applying fungicide and fertilizer were tested. The 3 media tested were i) forest soil only ii) a 1:1 mixture of sand and soil and iii) a 1:1 mixture of sand and charcoalized rice husk. The two fungicide treatments were i) Orthocide® 50 "Captan" applied first to the soil surface when seeds were sown and again 1 month afterwards and ii) no fungicide treatment. The two fertilizer treatments were i) 1 granule of Osmocote 14:14:14 per seedling module, every three months after germination and ii) no fertilizer treatment. The experimental design was randomized complete block design with three replications of each of the 12-treatment combinations with 100 seeds for each replicate (Table 5). Seeds were watered by hand using a fine spray bottle. Germination and survival were monitored weekly, and the experiment ended 30 days after the final germination event was recorded.

Table 5 Experimental design on seed germination trials.

Treatments	**Description**
T1	soil + no fungicide + no fertilizer (control)
T2	soil + no fungicide + fertilizer
T3	soil + fungicide + no fertilizer
T4	soil + fungicide + fertilizer
T5	soil and sand (1:1) + no fungicide + no fertilizer

Table 5 (continued).

Treatments	Description
T6	soil and sand (1:1) + no fungicide + fertilizer
T7	soil and sand (1:1) + fungicide + no fertilizer
T8	soil and sand (1:1) + fungicide + fertilizer
T9	sand and charcoalized rice husk (1:1) + no fungicide + no fertilizer
T10	sand and charcoalized rice husk (1:1) + no fungicide + fertilizer
T11	sand and charcoalized rice husk (1:1) + fungicide + no fertilizer
T12	sand and charcoalized rice husk (1:1) + fungicide + fertilizer

3.3.4.2 Propagation from cuttings. A low-cost technique was used for cutting experiments, developed at the ASEAN Forest Tree Seed Centre; Muak-Lek, Thailand (AFTSC), using closed plastic bags to retain high humidity, a so-called a non-mist propagation system (Kantarli, 1993). Cuttings were collected from both sexes of the six selected adult *Ficus* spp. tree species. Lateral branches were cut and mature and hardened branches selected. Each cutting was 4 nodes long (about 10-20 cm in length, depending on species), basal cuts at least 0.5 cm below a node, with only one leaf attached, and the leaf on each cutting was cut in half. The cuttings were put in a plastic bag and placed in a refrigerator (5°C) overnight to seal the wound and prevent bacterial infection. The basal ends of the cuttings were dipped in rooting powder hormone (Seradix; IBA≠3; 4-(Indol-3-yl) butyric acid). Cuttings were planted to half of their length (2 nodes) into black plastic bags (5 x 13 cm) filled with sand and charcoalized rice husk (1:1). The small plastic bags were then placed in larger plastic bags (60 x 90 cm; with one liter of water added), sprayed with water until the medium

was saturated. Plastic bags were closed firmly to prevent moisture loss and kept under 70% shade. After that, the plastic bags were opened weekly, for 5-10 minutes, to allow some moisture out, dead or yellowing leaves were removed. Plants were watered only when the soil surface dried or if there was no evidence of condensation on the inside of the plastic bag. Time to emergence of first shoot, root and survival was observed weekly. Once mature leaves had expanded, seedlings from both techniques were pricked out and potted into new containers (black plastic bags, 6.5 x 22 cm) using a medium of soil, peanut husk and coconut husk (2:1:1). Cutting experiments were conducted to assess the effects of cutting from 3 different positions of harvested branches (terminal shoot, middle and lower) and two rooting hormone treatments (rooting powder hormone; Seradix # 3; treated/not treated). A randomized complete block design was used for all treatments (Table 6), with three replications for each species. Each replicate consisted of 36 cuttings.

Table 6 Experimental design on cutting trials.

Treatments	**Description**
T1	upper + without rooting hormone
T2	upper + with rooting hormone
T3	middle + without rooting hormone
T4	middle + with rooting hormone
T5	lower + without rooting hormone
T6	lower + with rooting hormone

3.3.4.3 Seedling growth trials. Seedlings from both propagation types were tested the effects of light intensity and frequency of fertilizer application. A randomized complete block design was used with three replications of 4-treament combinations with 30 seedlings for each replicate (Table 7). Seedling growth (root collar diameter, height, canopy width and health) and survival rate were monitored monthly.

Table 7 Experimental design on seedling growth trials.

Treatments	Description
T1	full sun light + slow-release fertilizer, applied every 3 months
T2	full sun light + slow-release fertilizer, applied every 2 months
T3	under 70% of shade net + slow-release fertilizer, applied every 3 months
T4	under 70% of shade net + slow-release fertilizer, applied every 2 months

3.3.5 *Ficus* plantings. Three different methods were tested for establishing *Ficus* spp. trees i) direct seeding; ii) planting stock from cuttings; and iii) planting stock from seed. Three replicated blocks (each approximately 30 x 30 m) were established to compare the field performance of the three planting stock types of six *Ficus* species in disturbed habitats. Plots were prepared by weeding with hand tools about one week before planting. In each block, different planting stock types of the six *Ficus* species (30 individuals per block for each of the two planting stock types, and 60 units of *Ficus* seeds for direct seeding; one unit = 1 bamboo tube with 100 seeds) were planted randomly (except for direct seeding where seeds were sown in

rows in order to find them easily when monitoring), with a mean distance between plants of 1.5 meters. Fifty grams of fertilizer (NPK 15-15-15) was applied in a ring around each tree (but not for direct seeds). After planting out, plots were weeded and additional dose of fertilizer applied at 6 week intervals, during the rainy season for the first two years after planting. The planted trees were monitored for field performance (survival and growth rates) 3 times; i) immediately post planting ii) at the end of the first rainy season and iii) at the end of the second rainy season. Measurements included height (root-collar to highest meristem measured by measuring pole); root collar diameter (measured with Vernier calipers; canopy width (at widest point using a tape measure) and the health of the trees was scored on a scale of 0 (dead) to 3 (perfect health).

For direct seeding, seeds were sown on the surface of the soil but protected inside bamboo tubes 8 x 10 cm (half of the bamboo tubes were stuck in soil), in order to prevent seed movement due to wind and rain. Each tube contained about 100 seeds but was counted as one unit irrespective of how many seeds germinated. Germination was monitored at weekly intervals for 3 months. After expansion of the second true leaf pairs, seedlings were monitored for field performance, using the standard silvicultural and monitoring methods as for the other planting stock types.

3.4 Data analysis

3.4.1 *Ficus* phenology. Separate analyses were carried out for male and female trees of each species. A monthly percentage of each reproductive phase and each leaf index were calculated. Fig crop duration was determined as the time from the appearance of the first fig to the disappearance of all the figs from an individual tree.

The mean duration and frequency of crops for individual trees and at the population-level were then calculated. The prevalence of asynchrony of fig production was also calculated as the % of individuals in each species population which bore both receptive and releasing phase figs within their crowns, averaged across the whole study period. Correlation analysis (Pearson's Correlation) was performed for receptive (RCP) phase versus ripe or wasp releasing (RPP/RLP) phase of female and male tree; monthly rainfall versus leaf/fig initiation; monthly temperature versus leaf/fig initiation; and leaf flushing versus fig initiation. All tests were carried out at $P \leq 0.05$ significance level using SPSS (version 17.0). Mean diameters of figs at each developmental stage and mean number of seeds/wasps per fig were calculated from all the censuses. Also, external and internal traits of each developmental stage (color, odor, non-pollinator, wasp-predator, seed-disperser etc.) were recorded.

3.4.2 Figs and their associated wasps. *T*-tests were used to explore differences between the two sexes. ANOVA (LSD) was used to explore differences in seed production and foundress numbers among different habitats and seasonality.

3.4.3 *Ficus* propagations/plantings. Data were subjected to analysis of variance (ANOVA, MANOVA), *T*-test and least significant difference (LSD) or Scheffe test (where needed) at $P=0.05$ significance level. The median length of dormancy (MLD) was calculated from the germination times of all seeds which germinated. Overall success of germination/cutting trials was defined as the probability of germination/shooting multiplied by the probability of early seedling survival, converted to a percentage, i.e. the number of seedlings that could be potted from sowing 100 seeds or make 100 cuttings. The relative growth rates (RGR) were calculated using the formula:

$$RGR = ([\ln (G2) - \ln (G1)] / (T2-T1)) \times 365 \times 100$$

where G1 and G2 are the growth parameters (root collar diameter, height and canopy width) at the beginning (T1) and end (T2) of the sampling period (FORRU, 2008).

3.4.4 Cost evaluation. Cost per plant of each planting stock type was calculated for each stage of the process throughout the study period (1.5 years for direct-seeded and 2.5 years for both nursery-grown plants). Operational costs and labor requirements for activities from seedling production in the nursery to post-planting maintenance were recorded. These included all materials and labor costs associated with seed/cutting collection, planting stock production, transportation, site preparation, plantation establishment and maintenance. We used a rate of US$ 6.53 per day (8 hr) to calculate labor expenses.