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Estimation of Seed Production and Germination of Ficus hispida and Ficus racemosa

Ghvirly Ramadhani, Noor Farikhah Haneda^{*}, Iwan Hilwan

Department of Silviculture, Faculty of Forestry and Environment, IPB University. IPB Dramaga Campus, Bogor 16680, West Java, Indonesia

* Corresponding Author. E-mail address: nhaneda@apps.ipb.ac.id

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ABSTRACT

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Ficus hispida and Ficus racemosa are Moraceae species that commonly grow on riverbanks. Actions to propagate generative plants required more observation due to mutualistic symbiosis between F. hispida and F. racemosa with Ceratosolen solmsi and Ceratosolen fusciceps as pollinating insects. This study aimed to obtain data on seed production, germination, and the interaction of F. hispida and F. racemosa with pollinating insects. Research activities included seed extraction, seed germination testing, and calculation of the number of pollinating insects found in syconia. The results showed that the number of entered insects was related to syconia's receptive phase and diameter. F. hispida produced 3679 seedlings, while F. racemosa produced 2086 seedlings in one period. The number of seeds could cover a 6 ha area with a 4 m \times 4 m planting space. The germination percentage of F. hispida and F. racemosa was low to moderate. F. hispida seed germination was only 57.2% and F. racemosa was 68.6%. Thus, the seed should be germinated immediately after extraction.

1. Introduction

Ficus hispida and Ficus racemosa are Moraceae family species with many ecological roles. F. hispida, locally known as "bisoro", is a species found in the secondary forest close to freshwater sources such as rivers, lakes, and swamps (Berg and Corner 2005). Meanwhile, F. racemosa, locally known as "loa" is more commonly found growing naturally around rivers (Paarakh 2009). Both are species that produce fruit throughout the year so that they can support wildlife when other plants do not produce fruit (Lee et al. 2013).

In addition to their ecological role, F. hispida and F. racemosa are the well-known fig plant in the Indian Traditional System of Medicine with multiple Pharmacological actions (Yadav et al. 2015). Each part of F. hispida provides many benefits, such as giving a cool sensation to the human body, can be used as an astringent, relieving dysentery, and helping reduce bleeding from the nose or mouth (Rahman and Khanom 2013). The parts of F. racemosa used in traditional medicine include the bark, leaves, fruits, roots, latex, and seeds (Deep et al. 2013). F. racemosa, based on a review from Paarakh (2009), is known to have antioxidant, antidiarrhoeal, anti-inflammatory, antifungal, antibacterial, antifilarial, hypolipidemic, hepatoprotective, antipyretic, and hypoglycemic activities that can be beneficial for people with diabetes. Furthermore, the fruit of *F. racemosa* has aphrodisiac properties, while the stem bark has anti-hypertensive properties (Ajeigbe et al. 2021).

Up to now, *F. hispida* and *F. racemosa* are still classified as species that are "lesser-known" dan "lesser-used" in Indonesia. At the same time, the wood species can be used for rehabilitation in river buffer zones, lakeside areas (Soejono et al. 2013), and post-mining areas (Adman et al. 2012). The presence of *F. hispida* and *F. racemosa* in the urban environment may not be realized so that gradually it can threaten the existence of both species. Plant propagation efforts need to be done to preserve the presence of *F. hispida* and *F. racemosa* species. The generative reproduction of plants through seeds is a common and easy way to obtain large quantities of plant seeds. The generative reproduction of *F. hispida* and *F. racemosa* is a unique and distinctive event because the life cycle of the ficus plant cannot be separated from the interaction of these species with insects from the Agaonidae family (ordo: Hymenoptera) (Harrison 2005). These insects are in a mutualistic symbiosis with ficus plants because they need each other to complete their life cycle. Ficus plants can only be pollinated by pollinating insects Agaonidae, which can only reproduce in ficus flowers (Pothasin et al. 2014). The reproductive organs in ficus are a collection of female and male flowers surrounded by an enlarged flower base that resembles fruit. This structure is also known as syconia or fig (Harrison et al. 2000).

Ficus plants are divided into two types, namely monoecious ficus and dioecious ficus (Berg and Corner 2005). *F. racemosa* is one of the monoecious ficus species, so that this species will produce syconia consisting of male flowers, female flowers, and gall flowers. When ripe, the syconia will produce pollinated seeds and the pollinating insects *Ceratosolen fusciceps* that have reproduced can be found in it (Berg et al. 2011). *F. hispida* belongs to the dioecious ficus, so the male and female reproductive organs are located on different trees. Male trees will produce syconia consisting of male and gall flowers, and then when ripe, pollen and pollinating insects *C. solmsi* that has reproduced can be found in the syconia (Yang et al. 2013; Chen et al. 2018). The female tree is where syconia ficus seeds are produced, consisting entirely of female flowers (Weiblen 2000; Chen et al. 2018). The entire series of ficus reproduction requires special attention because information about the reproduction of these species is still limited. Therefore, this study aimed to obtain data on the production of *F. hispida* and *F. racemosa* seeds and the germination of these seeds.

2. Materials and Methods

2.1. Research Location and Object

The study was conducted in September 2017-February 2018 (five months). Samples of *F. hispida* and *F. racemosa* syconia were collected at the Dramaga Campus of IPB University, Bogor. Seed germination was carried out in the greenhouse of the Forest Ecology division, Department of Silviculture, Faculty of Forestry, IPB University. Observation and analysis of seed production data were carried out at the Forest Entomology Laboratory, Department of Silviculture, Faculty of Forestry.

The germination medium used was sterilized sand. The tools used are cutter, spoon, sieve, fabric filter, plastic container, analytical scale, petri dish, and germination tube. Data processing and analysis were performed using Microsoft Excel software.

(1)

2.2. *Methods*

2.2.1. Estimation of seed production

Estimation of seed production was carried out to obtain an estimate of the number and weight of seeds that could be produced from several syconia extractions. Syconia was chosen by the random sampling method. The number of syconia that would be extracted was 6 with a total weight of 100 g, with three trials. The extracted seeds were then weighed and the number of seeds obtained from 100 g of syconia was calculated. The extraction process is carried out by dredging the inside of the syconia, which contains seeds, using a spoon or cutter. The collected parts were sieved (the diameter size of the sieve used in this step was 28 cm with 1 mm of pore size) and washed with tap water to separate the seeds from other parts and remove the mucus adhering to the seeds. The clean seeds are then air-dried for 24 h until the seeds dry and separate from each other, making them easy to count and weigh (Effendi and Mindawati 2015).

2.2.2. Seed germination test

The seed germination test aimed to obtain data on the germination potential of *F. hispida* and *F. racemosa* seeds. Seed extraction was carried out following the guidelines from Effendi and Mindawati (2015) for the seed of Jiwai (*F. variegata*). The test was carried out on 100 seeds per germination tube with five trials so that the total number of seeds that had to be set aside was about 500 seeds. Ficus seeds are small. Therefore the seeds were sown in a germination container with a sterilized fine sand medium. Sprouts were placed in a greenhouse; maintenance includes watering with a fine spray. The calculation of germination was carried out 14 days after sowing by counting the number of seeds that germinated because, based on Effendi and Mindawati (2015), the seeds will start to germinate on the 11-14th day after sowing.

2.2.3. Interaction of ficus plants with pollinating insects

Observation of the interaction of ficus plants with insects was performed by counting the number of insects that entered the syconia of *F. hispida* and *F. racemosa*. The methods for counting the number of entered insects follow these steps: (i) Take syconia of *F. hispida* and *F. racemosa;* (ii) Cut the fruit crosswise using a cutter or knife; (iii) Note down the pollinating insects contained in syconia, only the number of pollinating insects is recorded, ignore other types. The number of insects that enter can be determined by dividing the *F. hispida* and *F. racemosa* syconia and then counting the number of insects contained in the syconia. Observations were started by taking several syconia of various sizes and then measuring their diameters transversely. The measurement results are divided into 10 classes (K) diameter, which will be determined through the following formula (Walpole 1995):

$$Data \ range \ (R) = Largest \ data - Smallest \ data$$
(1)

Interval length in class (P) =
$$\frac{R}{K}$$
 (2)

Determination of the upper limit of class = (lower limit value + P) - 1 (3)

Furthermore, each class interval will be compiled and calculated to get the frequency data. Each diameter class is represented by at least ten syconias with three trials. The diameter class becomes a reference in this study in observing interactions and grouping the number of insects.

2.3. Data Analysis

2.3.1. Germination percentage

The calculation of germination percentage refers to Sutopo (2002) with the following formula:

$$P = -\frac{n}{N} \times 100\% \tag{4}$$

where P is germination percentage, n is the number of germinated seeds, and N is the number of seeds germinated.

2.3.2. Peak germination value (PV)

The peak germination value was obtained from the maximum percentage of germination achieved by the seed at a certain time, with the formula:

$$PV = \frac{\% \text{ of germination on day-i}}{Number \text{ of days to germinate}}$$
(5)

2.3.3. Mean daily germination (MDG)

Mean daily germination value was obtained from the maximum germination value divided by the duration of the test, with the formula:

$$MDG = \frac{\% \text{ of germination at the end of the test}}{Duration of the test}$$
(6)

2.3.4. Germination value (GV)

The number of germination data was analyzed to obtain Germination Value (GV) with the following formula (Caliskan 2014):

$$GV = PV \times MDG \tag{7}$$

where GV is germination value ($[\%/day]^2$), PV is peak value (%/day), and MDG is mean daily germination (%/day).

3. Results and Discussion

3.1. Results

3.1.1. Seed production of F. hispida and F. racemosa

Estimation of seed production was carried out to estimate the number and weight of seeds that could be produced from a total of 100 g of syconia. **Table 1** presents data on the results of seed extraction and the estimated number of extracted *F. hispida and F. racemosa* seeds. The average yield of *F. hispida* seed extraction was 3.55 g, and it was estimated that there were 2263 seeds extracted from 100 g of wet syconia. The average weight of the extraction results of *F. racemosa* syconia is 0.98 g, with an estimated average number of seeds extracted as 8344 grains.

Based on **Table 1**, the result of *F*. *hispida* seed extraction was higher than *F*. *racemosa*. Moreover, the number of seeds is directly proportional to the weight of the extracted seeds.

Trials	Extracted s	seed weight (g)	Estimated number of seeds per 100 g of syconia		
	FH	FR	FH	FR	
1	3.40	0.81	12 593	7364	
2	3.46	1.23	11 162	10 250	
3	3.78	0.89	13 035	7417	
Mean	3.55	0.98	12 263	8344	

Table 1. Seed production of F. hispida and F. racemosa

Notes: F. hispida (FH) and F. racemosa (FR).

3.1.2. Seed germination of F. hispida and F. racemosa

Table 2 presents data on the average percentage of *F. hispida* seeds ranging from 45-66%, with an average of 57.2 %. The percentage of *F. racemosa* seeds ranged from 61-83%, with an average of 68.6%. The PV values of *F. hispida* and *F. racemosa* were quite varied, with an average of 2.96 and 4.66. However, the MDG values of both species did not vary much, with the average MDG value of *F. racemosa* being 4.90 and *F. hispida* being 4.09. Based on the calculation, the average seed value of *F. hispida* was 11.81, while the average seed value of *F. racemosa* was 24.61.

Repetition	Germination percentage (%)		PV		MDG		Germination value	
	FR	FH	FR	FH	FR	FH	FR	FH
1	71	66	2.5	3.7	5.07	4.71	12.68	17.29
2	64	65	4.7	2.5	4.57	4.64	21.33	11.61
3	61	60	2.1	1.9	4.36	4.29	9.20	8.14
4	64	45	2.3	5.0	4.57	3.21	10.67	16.07
5	83	50	11.7	1.7	5.93	3.57	69.17	5.95
Mean	68.6	57.2	4.66	2.96	4.90	4.09	24.61	11.81
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Table 2. Seed germination of F. hispida and F. racemosa

Notes: F. hispida (FH), F. racemosa (FR), Peak Germination Value (PV), mean daily germination (MDG).

3.1.3. Interaction of ficus plants with pollinating insects

The results of measurements of diameters and the number of pollinating insects found in *F*. *hispida* and *F*. *racemosa* are presented in **Table 3**. Diameter class 1 is the initial stage of syconia development, with an average diameter of male syconia *F*. *hispida* was 11.02 mm, the average diameter of female syconia *F*. *hispida* was 7.66 mm, and the average diameter of syconia *F*. *racemosa* was 8.10 mm. The diameter of the syconia continued to increase until the diameter class 10 (which was the mature stage of syconia), with the average diameter of male syconia was 40.24 mm, the average diameter of female syconia was 38.82 mm, and the average diameter of syconia *F*. *racemosa* was 44.07 mm. Insect pollinators found in diameter class 4 in each are a sign of the ongoing receptive phase of syconia. The calculated results in **Table 3** showed that the number of pollinating insects increased along with increasing the diameter of the syconia.

Diameter	AD of MFH	Number	AD of FFH	Number of	AD of FR	Number of
Class	(mm)	of insects	(mm)	insects	(mm)	insects
1	11.02	0	7.66	0	8.10	0
2	14.01	0	11.31	0	11.58	0
3	17.53	0	14.60	0	15.33	0
4	20.54	1	18.32	7	19.20	33
5	23.96	11	21.52	1	23.77	48
6	27.69	17	25.31	14	27.74	83
7	30.41	16	28.10	13	31.80	135
8	34.18	34	31.58	6	36.16	66
9	37.49	36	35.30	12	39.78	>100
10	40.24	9	38.82	5	44.07	0

Table 3. Diameter and number	of pollinating insects on	i syconia F. hispida a	and F. racemosa
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Notes: Average diameter (AD), male F. hispida (MFH), female F. hispida (FFH), and F. racemosa (FR).

3.2. Discussion

3.2.1. Seed production of F. hispida and F. racemosa

Table 1 show that the seed potential that can be produced from 100 g of syconia is relatively large. The seeds of *F. racemosa* were smaller than those of *F. hispida* (**Fig. 1**), but the weight and estimated number of F. *racemosa* seeds were less than *F. hispida*. It might be because the *F. racemosa* syconia consisted of male flowers, gall flowers, and female flowers, so the number of seeds produced by each *F. racemosa* syconia was less than *F. hispida*. The larger diameter and weight of *F. racemosa* were thought to be higher than *F. hispida*, thus affecting the wet weight of the weighed syconia and the number of fruits in 100 g of syconia. The number of *F. hispida* syconia in 100 g was five pieces, while the number of *F. racemosa* syconia was four pieces.



Note: Path= the length of the seed **Fig. 1.** Seed of *Ficus hispida* (right) and *F. racemosa* (left).

If one ficus tree can produce 500 - 1,000,000 syconia (Janzen 1979), the potential for ficus seeds produced in one fruiting period is enormous. Based on this, the estimated number of *F*. *hispida* seeds that can be produced in one period is around 355 g for each tree, while for *F*. *racemosa*, it is around 122 g for each tree. The yield of seed extraction from 100 g of syconia was estimated to be 12 263 grains for *F*. *hispida* and 8344 grains for *F*. *racemosa* (**Table 1**). Based on these data, if the number of seeds that germinated was only about 60% for *F*. *hispida* and 50% for *F*. *racemosa*, then the number of seedlings that could be weaned was 7358 and 4172 seedlings, respectively. This amount is estimated to be sufficient for seed needs if used for nursery purposes. The seeds of *F*. *hispida* and *F*. *racemosa* are considered recalcitrant seeds because of their inability to be stored for long periods like orthodox seeds because they will lose their viability (Berjak

2017). Thus, the seeds should be germinated immediately. Then if only half of that number can survive as seedlings, the potential number of *F. hispida* seedlings is 3679 seedlings and *F. racemosa* is 2086 seedlings. The number of seeds is estimated to be sufficient to be applied with a spacing of 4 m \times 4 m on 6 ha area for *F. hispida* and 3 ha for *F. racemosa*. The estimated number of seedlings is used to provide an overview of the utilization of *F. hispida* and *F. racemosa* seedlings for planting purposes.

3.2.2. Seed germination of F. hispida and F. racemosa

The percentage of *F. hispida* and *F. racemosa* germination rates was low to moderate. Based on the opinion of Sutopo (2002), seed germination is influenced by two factors, namely internal factors (seed maturity level, seed size, dormancy) and external factors (water, temperature, oxygen, light). This study used the same treatment for each replication, so it was assumed that the germination of the seeds tended to be influenced by internal factors, that is, seed viability. It might be caused the seeds did not germinate immediately after processing, so the germination of the seeds was reduced. The seed germination of *F. hispida* and *F. racemosa* seeds was thought to decrease with storage time, meaning that it was strongly suspected that the seeds were recalcitrant.

The metabolic process in recalcitrant seeds still takes place when the seeds are ripe, and the moisture content is still relatively high so that the life span of the seeds is shorter because the initiation of germination takes place immediately after the seeds are separated from their source (Pramono and Rustam 2015). Seeds should be germinated immediately because the germination percentage of *F. racemosa* seeds decreased greatly after being stored for six months. However, the germination percentage could be maintained up to 12.3% when the seeds were refrigerated for 12 months (Mathew et al. 2010). It showed that the ripe seeds of *F. hispida* and *F. racemose* should not be stored but must be germinated immediately after extraction.

The peak germination value (PV) indicates the maximum germination achieved by the seed at a certain time. This value indicates the vigor of the seed. Seeds have good vigor if the peak germination value is high enough, which is achieved in a relatively fast time. The PV of *F. hispida* and *F. racemosa* seeds varied, averaging 2.96 and 4.66. The higher PV of *F. racemosa* indicated that the vigor of *F. racemosa* seeds germinated at that time was better than that of *F. hispida* seeds. In addition, the PV varied in each replication, indicating a low seed vigor and non-uniform seed quality even though the seeds came from the same tree. Seeds with high vigor will germinate faster and seeds with the lowest vigor will appear later in the early phase of growth (Dwipa and Saswita 2017).

MDG (mean daily germination) is the average daily germination value that shows the ability of seeds to germinate every day. The MDG values for *F. hispida* and *F. racemosa* seeds were 4.09 and 4.90, which means that, on average, four seeds could germinate per day. The germination value is an index for the speed of seed germination. The higher the germination value, the better the seed germination process. Seed germination values of *F. hispida and F. racemosa* were 11.81 and 24.61. The germination value obtained in this study indicated that the germination process of *F. racemosa* seeds was generally better than that of *F. hispida* seeds. It might be because the seeds were germinated simultaneously but were extracted at different times and stored in inappropriate packaging of paper bags in a room without air conditioning. In addition, there is no pre-treatment before the seeds are germinated.

The seeds should be extracted when the fruit reaches physiological maturity because, at that time, the seeds have sufficient food reserves available to grow faster (Dwipa and Saswita 2017). In addition, before the seeds are germinated, pre-treatment should be carried out so that the seeds can germinate uniformly and more quickly (Schmidt 2002). If seeds are stored, their moisture content should be measured before storage because the viability and initial moisture content of the seeds greatly determine their shelf life of the seeds (Sutopo 2002). Packaging and storage procedures can be carried out following the recalcitrant seed storage procedure that the seeds are stored in airtight containers at a room temperature of 18-20°C with packaging and storage (Sudrajat and Pramono 2005).

Effendi and Mindawati (2015) stated that *F. variegata* seeds could be packaged in aluminum foil containers in a room with an air conditioner to maintain the seed viability for up to 6 months. Paramita et al. (2018) explained that indoor storage with an air conditioner is better in maintaining moisture content, viability, and seed vigor than storage at room temperature. Based on this, the recommended storage methods for *F. hispida* and *F. racemosa* seeds are: (a) maintaining seed moisture content ranging from 20-50%, (b) packing seeds in airtight containers such as aluminum foil, (c) then storing the container in the refrigerator or refrigerated room so that it can be stored longer.

3.2.3. Interaction of ficus plants with pollinating insects

The results of the estimated number of seeds test (**Table 2**) showed that the number of seeds obtained from 100 g of syconia *F. racemosa* was less than *F. hispida*. This tendency is related to the composition of the ficus flower in one house, which is located in one syconium, so that the space in the syconia is divided for the ficus to produce seeds and regenerate pollinating insects. Harrison and Yamamura (2003) stated that the monoecious ficus is less efficient in terms of nutrient use because about 50% of the ovules are "damaged" due to use by pollinating insects as breeding grounds. *F. hispida* is a ficus with two houses, so it has female syconia, which will only produce seeds. When noted from the point of view of ficus importance, the dioecious ficus is more efficient because insects use no ovaries. Pollinating insects can experience a dilemma against male and female syconia, but if pollinating insects enter the male syconia, the pollinating insects will have the opportunity to produce more offspring.

Yang et al. (2002) found that the number of insects that entered the *F. hispida* syconia was related to the number of seeds and galls that would develop. The number of pollinating insects that enter the female syconia of *F. hispida* generally amounts to 1 or 2 individuals, with the maximum number found in four individuals. When one individual pollinating insect enters one female syconium, the percentage of seeds to the total number of female flowers is 51.3%. When 2 or 3 individual pollinating insects enter one female syconium, the percentage of seeds to about 86.5%. However, when there are four or more pollinators, the percentage of seeds decreases by around 40.2% to 63.8%. It happened because the pollinating insect's oviposition ability to decrease.

Yang et al. (2002) showed that the number of insects entering the syconia is related to the condition of ongoing receptive syconia. If there are many syconia in the receptive phase, the number of insects that enter one syconium varies between 2-3 individuals. However, if there are a few syconia in the receptive phase, the number of individual pollinating insects that enter one

syconium can reach 5-9 individuals. In this study, the number of insects found in *F. hispida* and *F. racemosa* was generally 1 or 2, and the maximum number found was four individuals. The diameter of the monoecious syconia ficus is known to affect the number of species and insect populations, so it can be said that the larger the diameter of the syconia, the greater the availability of resources for both ficus and pollinating insects. (Cook and Segar 2010; Castro et al. 2015; Jauharlina et al. 2017).

4. Conclusions

The results showed that the seeds that could be produced by *F. hispida* and *F. racemosa* in one period were relatively high when applied for nursery or planting purposes. However, the seed germination percentage of *F. hispida* and *F. racemosa* was not very high and ranged from 50-70%. The abundance of seeds to be produced is related to the size of the syconia diameter and the number of insects that enter the syconia. Seeds of *F. hispida* and *F. racemosa* are recalcitrant, so they should be germinated immediately. If seeds need to be stored, it is necessary to use the right way to store them to maintain their viability. Syconia collecting activities should be carried out when the fruit (syconia) has shown signs of fruit ripening to obtain good quality seeds. Research on the reproductive biology of *F. hispida* and *F. racemosa* needs to be continued to obtain information related to the stages of syconia development based on the period and season in Indonesia. In addition, further information is needed regarding the seed germination of wild animals on seed germination.

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