

# Ecophysiology of *Lophira lanceolata* Seeds Germination and Conservation Perspectives

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## Abstract

*Lophira lanceolata* is a multi-purpose woody plant species used by local populations in Benin. Its seeds are used for the manufacture of edible vegetable oil and also for medicinal care. However, reproduction by seed of this plant species is compromised not only by the multiple uses made of the seeds, but also by the difficulties of germination in the natural environment. Several ecological factors could explain this difficulty of germination. In this study, we investigated the species seeds ecophysiology in order to develop seedlings production techniques for its reintroduction. Beta regression was performed to test the effect of water, light and provenance on seeds germination rate. The germination speed was expressed as the median germination time. The results showed that the germination rate was better under light conditions ( $\beta = 0.77674 \pm 0.14954$ ,  $Z = 5.194$ ,  $p < 0.0000$ ). The germination rate decreased as the frequency of watering increased ( $\beta = -0.28222 \pm 0.14809$ ,  $Z = -1.906$ ,  $p = 0.0567$ ). The best provenance was the phytodistrict of Atacora chain. The cumulative germination rate reached 50% after 54 days for seeds collected from Atacora chain and after 57 days under light conditions. Regarding the level of watering, the germination rate remained below 50% regardless the treatment. The germination speed was higher for seeds collected from Atacora chain and that have germinated under light conditions and watered twice a week. For the sustainable management of *L. lanceolata*, domestication by establishment of plantations by rural communities is recommended based on seeds from Atacora chain.

## Keywords

Benin, Germination Speed, Oilseed, Phytodistrict, Recalcitrant Seed, West Africa

## 1. Introduction

*Lophira lanceolata* Tiegh. ex Keay, also known as False Shea, is a sudanian and sudano-guinean savannas species in Central and West Africa [1]. It is a meso-phanerophyte, often gregarious, on sandy or gravelly soils [2], up to 700 m altitude in northwestern Benin [3]. In Benin, *L. lanceolata* can reach 19 m height and a diameter at breast height of 58.2 cm with stalked leaves, simple, complete and alternate but grouped at the end of branches [4]. Its inflorescence is a terminal panicle, pyramidal, 15 to 20 cm long. The flowers are bisexual, regular and white in color. The fruits are assimilated to a conical form. The seeds are ovoid, brown in color and glabrous [2].

Wood of *L. lanceolata* is very used for charcoal and as firewood [5]. The leaves, bark and roots are used in the treatment of various pathologies such as constipation, flatulence, chronic wounds, menstrual pain, gastric pain [2], hemorrhoids [6], cough [7], erectile dysfunction and sexual activity [8], stomach pain [9], jaundice [5], diabetes [10], malaria [6] [9], etc. The oil extracted from the seeds, is rich in polyunsaturated fatty acids [11] and is used in human food, cosmetics and preparation of traditional soaps [12].

This species undergoes multiple disturbances across its range, which negatively affects its survival. It is increasingly difficult to harvest the seeds. Besides, adverse ecological conditions have a negative impact on the development and growth of the regeneration of this species in its natural habitats [13]. Moreover, in some sites, despite the high fruit productivity of *L. lanceolata*, seminal regeneration appears very weak, in response to anthropogenic pressures and seeds collection for edible oil production [14]. In addition, fruits and seeds of *L. lanceolata* are often attacked by pests whose main insect identified in Burkina Faso are *Ephestia* spp., *Tribolium castaneum*, *Oryzeaphilus* spp., and *Tenebroides mauritanicus* [15].

Although natural regeneration by seedling is the main mode of species reproduction [16], some species such as *L. lanceolata* have low germination rates including their natural germination by seeds [14]. In order to elaborate silvicultural and conservation actions of *L. lanceolata*, factors governing seeds germination and seedlings growth should be investigated. Unlike orthodox seeds, which can be easily stored under artificial conditions, *L. lanceolata* seeds have low humidity and generally have varying degrees of dormancy [17]. Its seeds are recalcitrant and because of their moisture content and sometimes high oil content they are difficult to store. However, when seeds are harvested fresh, they germinate and establish themselves easily in the nursery and also germinate abundantly in natural formations [17]. According to [18], temperature plays an important role in the germination initiation process. It acts mainly on the speed of germination [19]. For oilseeds species such as *Pentadesma butyracea*, the action of heat appears to be detrimental to germination because of their high oil content [20]. Soil moisture content also plays an important role in seed germination [21]. In addition, light intensities significantly affect seed germination [19]. For many

seeds, temperature fluctuations can be substituted by the need for light [22]. The interactive effect of water, light and temperature on the germination of oilseeds has been poorly investigated and is still a subject of interest in the ecology and silviculture of multipurpose woody species [20]. Leblanc (1998) and Natta (2013) argued that, it is the interaction of these factors, in a synergistic, antagonistic or additive manner that triggers germination. Germination of a seed is generally characterized by its delay, the phasing out of dormancy and the germination rate [23]. Differences in germination characteristics as a function of climatic provenances are also commonly observed for widely distributed plant species [24].

This study explored the influence of station conditions on seed germination of *L. lanceolata* in Benin. Specifically, it aimed to assess the effect of seeds provenance, watering frequency and light conditions on the germination rate and speed of *L. lanceolata* seeds. It was generally assumed that the germination rate and speed of *L. lanceolata* seeds vary according to the provenance and light conditions. We hypothesized that the germination rate and speed of *L. lanceolata* seeds is significantly higher for more watered seeds, receiving sufficient light and coming from the phytodistrict of Atacora chain.

## 2. Material and Methods

### 2.1. Study Area

Germination tests of *L. lanceolata* seeds were carried out at the experimental farm of KIKA Agricultural High School (Commune of Tchaourou, Central East Benin, 8°53'00"N and 2°36'00"E). It is the largest administrative district in Benin (7256 km<sup>2</sup>) with a Sudano-Guinean climate. The average annual rainfall varies from 1100 to 1200 mm per year with an average temperature between 26°C and 30°C. The hottest weather takes place in February, while the coldest occurs in August. Relative humidity varies from 15% in the dry season (January-February) to 99% in the rainy season (August-September) while the average annual insolation varies between 2200 and 2400 hours [25].

Seeds from ripe fallen fruits were collected in the phytodistricts of Atacora chain, Borgou-North and Borgou-South and were sown in polythene bags arranged in random blocks and on raised bed. The experiment lasted 72 days and 3 key factors (phytodistrict, watering and shading), specific to the ecology of the species were examined.

### 2.2. Experimental Design

Three levels of shading conditions namely: Without shading (WS) representing the control, Medium shading (MS) or 50% recovery and dense shading (DS) or 90% recovery were tested (Table 1). Similarly, three levels of watering were evaluated namely: without watering, watering once a week (11 L/bed per week) and watering twice a week (22 L/bed per week). Indeed, we used in this experiment watering cans having a capacity of 11 liters [20]. The watered seeds were used to test the environment in which natural populations of *L. lanceolata* live and develop, while the non-watered seeds constitute the controls. So, each level of

**Table 1.** Experimental design for *L. lanceolata* seeds germination.

Provenance	DS			MS			WS			Total
	WW	W1	W2	WW	W1	W2	WW	W1	W2	
Atacora chain	63	63	63	63	63	63	63	63	63	567
Borgou-North	30	30	30	30	30	30	30	30	30	270
Borgou-South	36	36	36	36	36	36	36	36	36	324
Total	129	129	129	129	129	129	129	129	129	1161

**Note:** DS: Dense shading; MS: Medium shading; WS: Without shading; W1: Watering once a week; W2: Watering twice a week; WW: Without watering.

shading was associated to three levels of seeds watering. A total of 1161 *L. lanceolata* seeds were sown and monitored in this experiment.

### 2.3. Data Analysis

The germination rate (GR) was calculated at different dates using the formula:

$$\text{GR (\%)} = 100 \times \text{Number of sprouted seeds} / \text{Number of sown seeds} \quad (1)$$

Beta regression [26] was performed in R 4.0.2 with betareg package [27] to test the effect of watering, shading and provenance on *L. lanceolata* seeds germination. Several models were parameterized and compared using the Akaike Information Criterion [28]. Models with  $\Delta\text{AIC} < 2$  were considered better [29].  $\Delta\text{AIC} = \text{AIC}_i - \text{AIC}_{\text{min}}$  is the difference between the AIC of a given model *i* and the smaller AIC. Considering the parsimony rules, the model that has the smallest AIC value and smallest number of terms influencing seed germination of *L. lanceolata* was preferred. It turned out that the best model was the one that reflects the additive effect of the different factors.

The germination speed (Gs) was expressed as the median germination time (Scott *et al.*, 1984), which formula is:

$$\text{Gs} = \text{T1} + (\text{T2} - \text{T1}) \times (0.5 - \text{G1}) / (\text{G2} - \text{G1}) \quad (2)$$

where:

G1 = cumulative percentage of sprouted seeds whose value is closest to 50% by lower value.

G2 = cumulative percentage of sprouted seeds whose value is closest to 50% per higher value.

T2 – T1 = Interval of the middle class.

Sprouted seeds were counted daily to determine germination capacity. They were assumed to have sprouted when the radicle pierces the integument [30].

## 3. Results

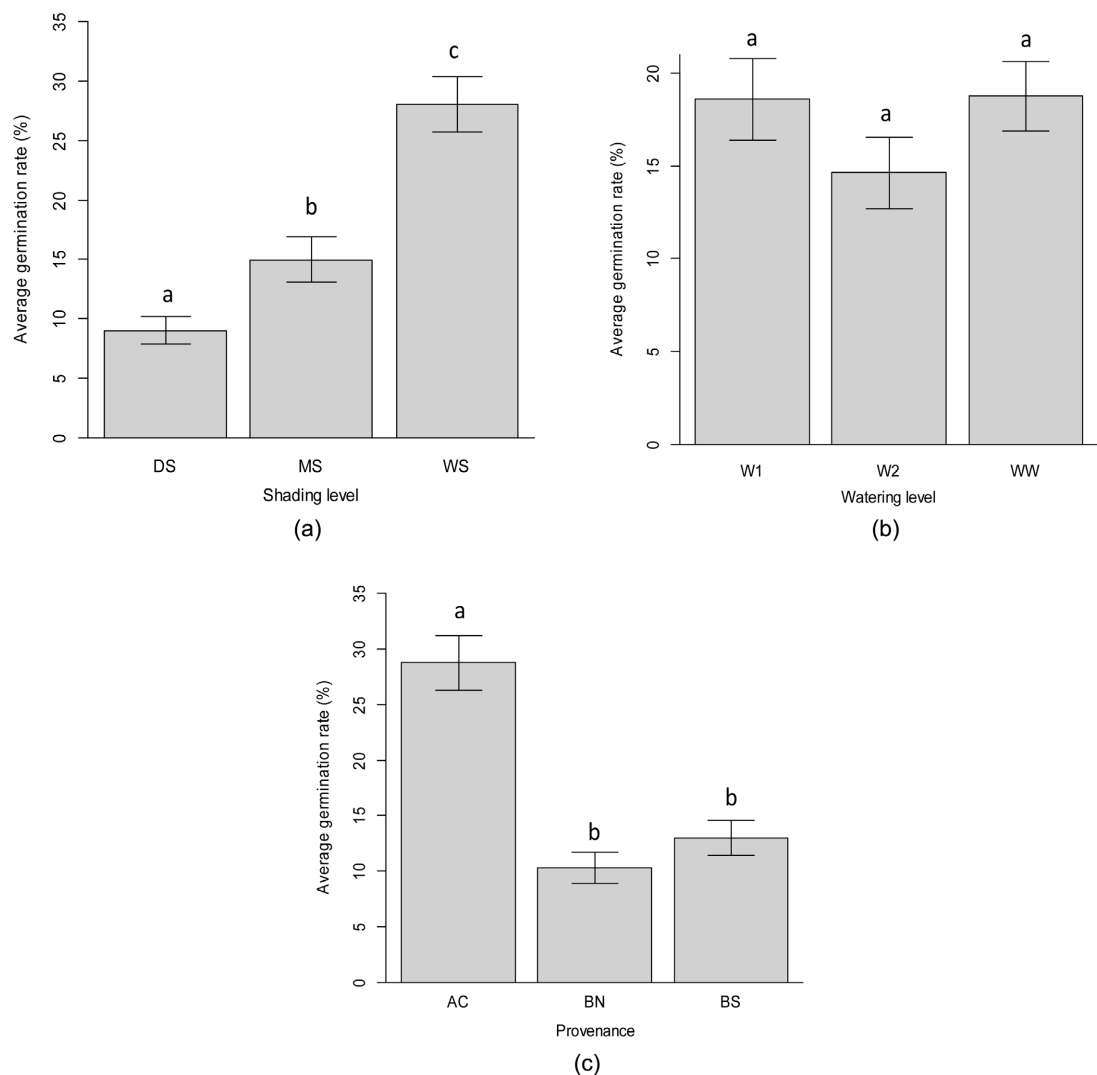
### 3.1. Effect of Watering, Shading and Seeds Provenance on the Germination Rate of *L. lanceolata*

Germination of *L. lanceolata* seeds was significantly influenced at 5% level by

shade level, and seeds provenance (Table 2). A 50% reduction in shading led to a higher germination rate ( $\beta = 0.31458 \pm 0.14736$ ,  $Z = 2.135$ ,  $p = 0.0328$ ) and this germination was better without shading conditions ( $\beta = 0.77674 \pm 0.14954$ ,  $Z = 5.194$ ;  $p < 0.001$ ) (Table 2, Figure 1(a)).

This study also showed that frequency of watering did not have a significant effect on germination rate of *L. lanceolata* seeds. However, this germination rate decreased as the watering frequency increased ( $\beta = -0.28222 \pm 0.14809$ ,  $Z = -1.906$ ,  $p = 0.0567$ ) (Table 2, Figure 1(b)).

Compared to seeds from Atacora Chain (Table 2, Figure 1(c)), the germination rate was significantly lower for seeds from Borgou-South phytodistrict ( $\beta = -0.71068 \pm 0.15009$ ,  $Z = -4.735$ ,  $p < 0.0000$ ) and those from Borgou-North phytodistrict ( $\beta = -0.74952 \pm 0.15008$ ,  $Z = -4.994$ ,  $p < 0.0000$ ).



**Figure 1.** Evolution of the average germination rate according to (a) shading levels, (b) watering and (c) seeds provenance. **Note:** BS = Borgou-South, BN = Borgou-North, AC = Atacora chain, WS = Without shading, MS = Medium shading, DS = Dense shading, WW = Without watering, W1 = Watering once a week, W2 = Watering twice a week. For each figure, the same letter ((a)-(c)) indicate that there is no significant difference.

**Table 2.** Test of the effect of watering, shading and provenance on *L. lanceolata* seeds germination.

Modalities	Estimate	Std. Error	Z value	Pr (> z )
(Intercept)	-1.56713	0.17042	-9.196	<2.00e-16***
Shade DS			Reference	
Shade MS	0.31458	0.14736	2.135	0.0328*
Shade WS	0.77674	0.14954	5.194	2.06e-07***
Watering W1			Reference	
Watering W2	-0.28222	0.14809	-1.906	0.0567.
Watering WW	0.03878	0.14880	0.261	0.7944
Provenance AC			Reference	
Provenance BS	-0.71068	0.15009	-4.735	2.19e-06***
Provenance BN	-0.74952	0.15008	-4.994	5.91e-07***

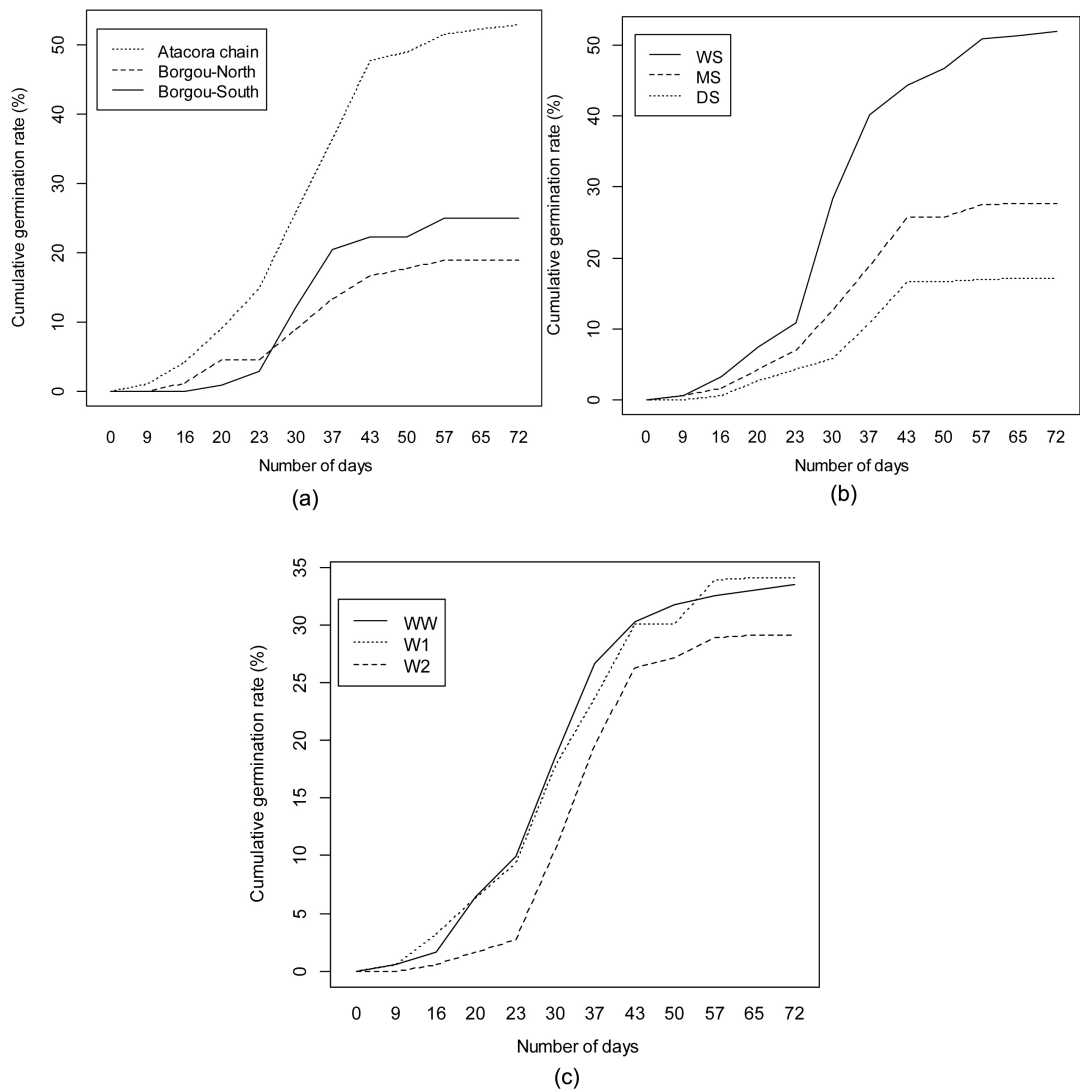
**Note:** DS: Dense shading; MS: Medium shading; WS: Without shading; W1: Watering once a week; W2: Watering twice a week; WW: Without watering, AC: Atacora chain, BS: Borgou-South, BN: Borgou-North. Asterisks show significant level: \* probability value  $p < 0.05$ ; \*\*\* probability value  $p < 0.001$ .

### 3.2. Germination Speed of *L. lanceolata* Seeds

**Figure 2** shows the evolution of the cumulative germination rate. It reached 50% after 54 days for the seeds collected from Atacora Chain while it remained below this threshold throughout the experiment for the seeds collected from Borgou-South and Borgou-North (**Figure 2(a)**).

In the absence of shade, the cumulative germination rate reached 50% after 57 days while it remained below this threshold throughout the experiment period when seeds were sown under medium shading (MS) or dense shading (DS) conditions (**Figure 2(b)**). However, regarding the level of watering, the germination rate of *L. lanceolata* seeds remained below 50% whatever the treatment and throughout the experiment (**Figure 2(c)**).

We found that the best model testing the effect of watering, shading and provenance on *L. lanceolata* seeds germination is the one that reflects the additive effect of the three factors (smallest value of  $\Delta AIC$ ). Before the end of the experiment (72 days), the germination rate reached a value greater than 50% for many experimental designs (**Figure 3**). The germination rate is higher in the experimental design consisting of seeds from Atacora chain, watered twice a week and tested in the absence of shade. However, the germination rate is lower for the seeds collected from Borgou-north, watered once a week and tested in the absence of shade (**Figure 4**).

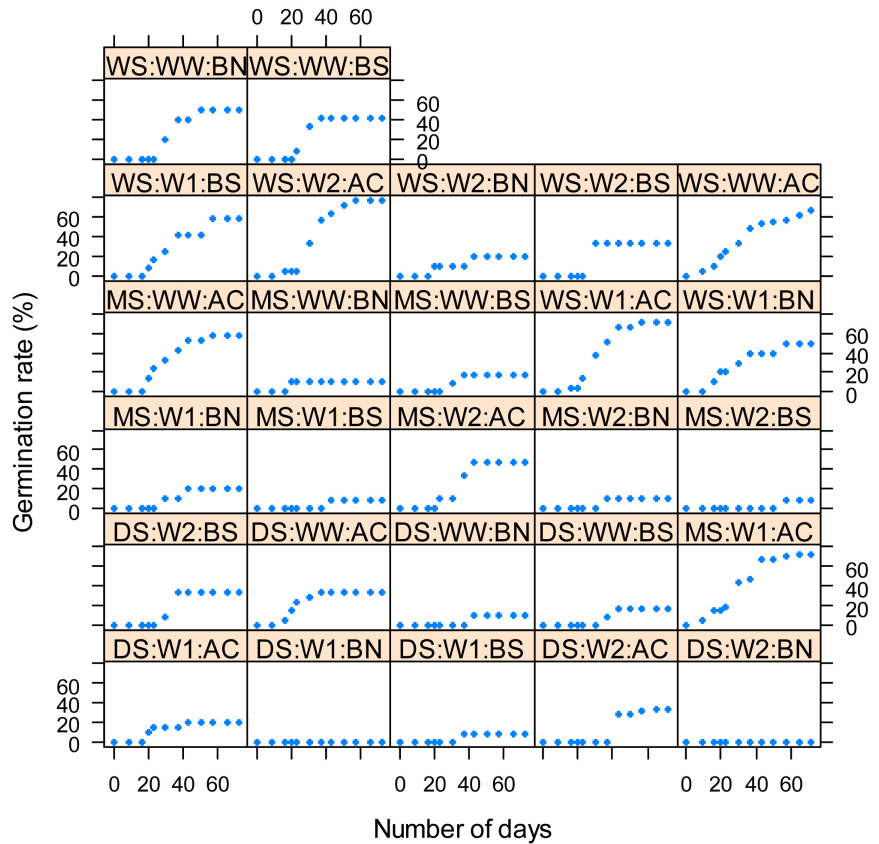


**Figure 2.** Evolution of the cumulative germination rate as a function of the number of days after sowing. **Note:** WS = Without shading, MS = Medium shading, DS = Dense shading, WW = Without watering, W1 = Watering once a week, W2 = Watering twice a week.

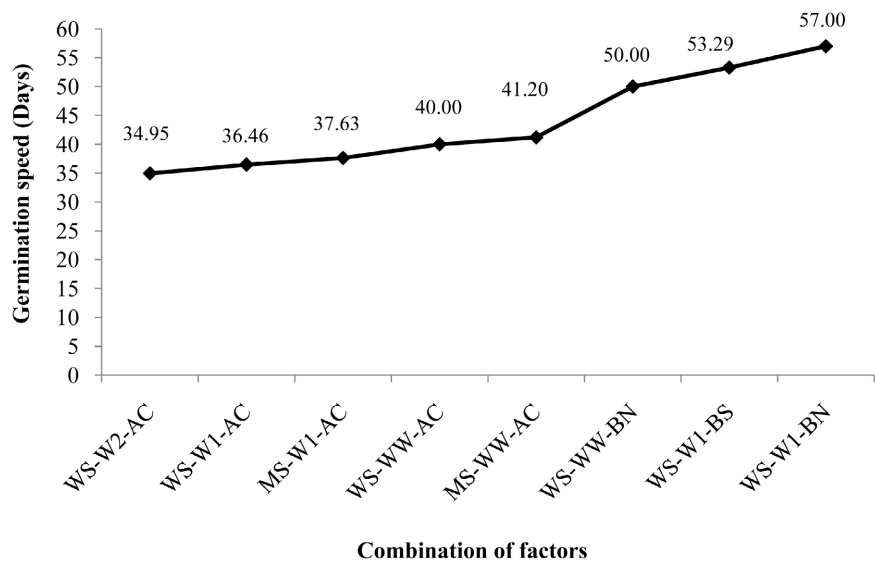
## 4. Discussion

### 4.1. Effect of Watering, Shading and Seeds Provenance on the Germination Rate of *L. lanceolata*

Germination of *L. lanceolata* seeds was significantly influenced by shade level, and seeds provenance. A 50% reduction in shading led to a higher germination rate and this germination was better without shading conditions. In fact, need for light is probably a genetic characteristic and can be mediated by phytochrome [31], which stimulates synthesis of growth promoting substances to initiate germination [32]. According to [33], sunshine is also an indispensable factor for good germination of seeds. The effect of light on the germination rate of *L. lanceolata* seeds is remarkable 9 days after sowing and seeds without shading germinate better. *L. lanceolata* is a savanna species [3] that sprouts under a very low



**Figure 3.** Cumulative germination rate according to combinations of factors and as a function of time. **Note:** BN = Borgou-North, BS = Borgou-South, AC = Atacora Chain, WS = Without Shading, MS = Medium Shading, DS = Dense Shading, WW = Without Watering, W1 = Watering once a week, W2 = Watering twice a week.



**Figure 4.** Evolution of the germination rate according to the combined factors. **Note:** BN = Borgou-North, BS = Borgou-South, AC = Atacora Chain, WS = Without Shading, MS = Medium Shading, DS = Dense Shading, WW = Without Watering, W1 = Watering once a week, W2 = Watering twice a week.



level of shade unlike forest gallery species for which shading and additional water supply, create a microclimate stimulating the lifting of seed dormancy [20]. In the case of *L. lanceolata*, we noticed under dense shading several cases of seed rot, the appearance of molds and the attack of microorganisms. Effects of increasing canopy opening on seed germination are somewhat species-specific, but the most common response is decreased germination in an open environment [17]. Seeds with high water content can be destroyed by relatively low moisture losses [34]. [35] reported that seeds of *Triglochin maritima* and *T. palustre* germinate in both light and darkness. According to [21], oilseeds germinate less under the action of heat. Unlike seeds of *P. butyracea* that germinate well under dense shading conditions [20] those of *L. lanceolata* have high germination rates in light environment like *Musanga cecropioides*, *Nauclea diderrichii* and *Milicia excelsa* that germinate well under light [17]. Light alone can control some species germination but it can also interact with temperature [35]. The interaction between temperature and light on germination should be studied as suggested by [35], because temperature was not fully controlled in our experiment. They reported that at a constant temperature of 30°C, light reduced germination rate to 3% compared to 37% in the dark, but that at alternating temperatures of 30°C/20°C, germination under light and shade was similar. All the seeds used in our experiment were freshly collected and generally showed good viability. [36] reported that most tropical species germinate freely when seeds are newly harvested including most pioneers. They also reported that dormancy can be imposed or induced later (or viability lost) if germination is prevented by lack of water, storage or dispersal in the shade of forest with low light. We did lack information on these processes concerning our species, and further investigations are needed to better understand the discrepancy observed in the germination rates.

This study also showed that frequency of watering did not have a significant effect on germination rate of *L. lanceolata* seeds. However, this germination rate decreased as the watering frequency increased. This could be explained by the fact that the experiment took place in the rainy season and an excess of water rot seeds and promotes a microclimate for pathologies and pests [37].

Compared to seeds from Atacora Chain, the germination rate was significantly lower for seeds from Borgou-South phytodistrict and those from Borgou-North phytodistrict. This suggests higher ability of seeds from the Atacora-Chain to germinate, even when the other provenances show environmental conditions (*i.e.*, the experiment was conducted in the Borgou-South phytodistrict). For species with a wide distribution range, differences in germination characteristics depending on the climatic zone of seeds are generally observed [24] [38] [39]. Variations among populations are probably due to the presence of different ecotypes with different germination strategies [24]. However, this hypothesis needs to be tested. Additional information could be gathered if the experiment was replicated in different climatic provenances of the seeds. In our study, the

percentage of germination was generally higher for *L. lanceolata* seeds collected from the phytodistrict of Atacora chain. This could be the result of favorable ecological conditions for *L. lanceolata* seeds germination in this phytodistrict. In fact, the phytodistrict of Atacora chain is predominated by mineral and little evolved soils which are favorable for the seed's germination. In addition, breeding trees in this phytodistrict reach their first age of reproduction at younger age (small diameter and height) and produce few heavy fruits per tree unlike the phytodistricts of Borgou North and Borgou-South [4]. According to several studies [15] [33] [40] [41], morphological traits variation of seeds and breeding trees can also affect seed germination. [24] [42] reported that maternal factors, such as the position of the seed in the fruit/tree and the age of breeding trees, influenced seeds germination capacity. However, we did not assess this effect in our study. Otherwise, the discrepancies observed in the current study regarding the provenance of seeds could be the result of a climatic conditions-driven genetic differentiation among the three provenances. [43] showed for 12 African provenances of *Faidherbia albida* that genetic variations related to seed traits were likely to influence seeds germination. [44] also found differences in the germination of *Caesalpinia bonduc* seeds from Benin and Togo. Similarly, [45] noted differences in germination rates of *Tectona grandis* between the agro-ecological zones of Benin. These differences in seeds germination rates between provenances can be explained by several factors, including: climatic, edaphic and genetic conditions.

#### 4.2. Germination Speed of *L. lanceolata* Seeds

The cumulative germination rate reached 50% after 54 days for the seeds collected from Atacora Chain while it remained below this threshold throughout the experiment for seeds collected from Borgou-South and Borgou-North. This difference in seeds germination capacity could result from a genetic differentiation between the three provenances. [4] observed that the *L. lanceolata* trees from Atacora chain phytodistrict enter fructification precociously and this could also affect the germination potential of seeds. The speed and uniform germination of seeds, followed by the rapid emergence of seedlings, are highly desirable characteristics in seedling production [46]. The assessment of germination depends not only on the percentage of germination scored, but also on the germination speed and its evolution over time [47]. These two factors combined are often used to determine the success of a treatment on seeds germination [48].

In the absence of shade, the cumulative germination rate reached 50% after 57 days while it remained below this threshold throughout the experiment period when seeds were sown under medium shading (MS) or dense shading (DS) conditions. The speed of germination also varied according to the species. For 335 species of Malaysian trees, Ng (1980) [49] showed that the time needed to complete germination varied between one week and more than one year. In some species such as teak, non-germinated (but still viable) seeds still maintain their

viability and germinate in the subsequent years when conditions are favorable [50]. This is not the case for *L. lanceolata* seeds that quickly lose their viability after 4 months of storage [4] due to their oleaginous nature [51]. Germination is an important step that includes the imbibition and recovery of the seed metabolism and ultimately the emergence of the embryonic radicle [52]. This metabolic recovery, emergence of the radicle, and preparation for subsequent seedling growth involve synthetic and protective processes involving components initially stored in the seed [53]. [54] proposed that tropical tree species could be divided into two guilds (functional groups, sensu [55]), pioneers and non-pioneers, distinguished by pioneers' dependence on the hole in the canopy for seeds germination and seedlings. Recalcitrant seeds are characteristic of non-pioneer species (climax), while orthodox seeds are associated with pioneer species (sensu [54]), which include relatively few forest tree species [56].

Water is an important factor in the speed of seed germination [20]. Some seeds germinate well and quickly only under conditions of sufficient humidity [18], as it is the case of *Neocarya macrophylla*, an oilseed species [21] like *L. lanceolata*. However, regarding the level of watering, the germination rate of *L. lanceolata* seeds remained below 50% whatever the treatment and throughout the experiment. According to [32] germination speed is influenced by watering, but this condition is not sufficient because the temperature must also be suitable and the embryo must be properly oxygenated.

Before the end of the experiment (72 days), the germination rate reached a value greater than 50% for many experimental designs. The germination rate is higher in the experimental design consisting of seeds from Atacora chain, watered twice a week and tested in the absence of shade. However, the germination rate is lower for the seeds collected from Borgou-north, watered once a week and tested in the absence of shade. In fact, seed germination is under the influence of several ecological factors [21] [57] [58]. We get more understanding of seeds germination when the three factors effect is combined in an interactive, synergistic, antagonistic or additive way [18]. According to [17], seeds germination speed also depends on their harvest at different times of the year, resulting from a very crucial biological property for adaptation to environmental constraints. For the case of *L. lanceolata*, seeds used for the germination in this study were harvested at the same period.

## 5. Conclusion

This study has investigated the influence of watering, shading and provenance on the germination rate and germination speed of *L. lanceolata* seeds. The best germination rate was observed for seeds collected from Atacora chain phytodistrict, grown in the absence of shade, and watered twice a week. Our results may be considered as baseline information to define conservation strategies of highly harvested oleaginous species such as *L. lanceolata*. However, further studies on other key variables of recalcitrant seed germination and growth monitoring are

needed for the species domestication. Further frontiers to be explored in the germination ecology of recalcitrant seeds include cellular and molecular studies of *L. lanceolata* seeds germination and research for optimal conditions for this multiple-use species seeds germination.

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### Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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