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Dehydration capacity and germination of the generative seeds of *Syzygium guineense* subsp. *macrocarpum* (Myrtaceae) in Benin.

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Abstract

The germinative power of recalcitrant seeds decreases as their moisture content decreases. This study aimed to verify the germination capacity of *Syzygium guineense* subsp. *macrocarpum*, a species of high honey value, in view of its domestication in Benin. The research was first conducted in a natural environment, particularly in the forest Bassila for plant material collection and regeneration monitoring, and then at the University Abomey-Calavi for moisture content determination, and germination test and seedlings growth. The initial water content of different categories of fruits was 55.70 ± 8.70% and 72.90 ± 7.78% (based on fresh weight). The mean fresh weight of a ripe fruit was 4.64 ± 1.53 g while its dry weight was 1.19 ± 0.31 g. Fresh seed weighted 2.10 ± 0.67 g while its dry weight measured 0.92 ± 0.32 g. This confirmed the recalcitrant behavior of these seeds, which displayed a germination rate of 32 to 42% after 25 days storage. The brittle and permeable integuments of *S. guineense* seeds did not protect them from dehydration. Immediate sowing is recommended to reach 80 to 90% germination for this species. Conservation measures should involve the population and forest users such as beekeepers and foresters in the development of the nursery of *S. guineense*.

Keywords: *Syzygium guineense*, seeds, germination, regeneration, Benin.

Introduction

Syzygium guineense (Willd.) DC. (Myrtaceae) has a wide distribution in tropical Africa (Akoègninou *et al*., 2006, Eyog Matig, 2006; Arbonnier, 2008; Maroyi 2008), locally gregarious in the Guineo and Guineo-Sudanian zones (Arbonnier, 2008). The tree finds many applications in traditional African medicine. Seed multiplication is easy and is the commonly used multiplication method (Maroyi, 2008). According to Maundu *et al*. (1999), *S. guineense* is a tree of which sweet edible fruits are used as drinks. It is an important bee fodder but its seeds are recalcitrant, highly significant desiccation-sensitive (Dagnachew, 2008) and must be sown immediately after harvest (Eyog Matig, 2006; Orwa *et al*., 2009; Maroyi, 2008).

Seed viability and seedling resistance to multiple natural aggressions can be determined by biological and ecophysiological factors specific to the species themselves (Willan, 1992; Lopez *et al*., 2000). They are essential determinants of seminal regeneration of plant species in a natural environment (Bationo *et al*., 2001). This is the case, for example, for the water content of seeds, which affects the longevity and storage conditions of seeds (Orozco-Segovia & Vazquez-Yanes, 1993). Similarly, the functional morphology of the seedlings affects their adaptability to constraining factors of the natural environment (Gorse, 1994; Ouedraogo & Alexandre, 1994; Sambou *et al*., 1994).

The seeds of the different plant species are mainly divided into three types depending on their ability to withstand desiccation: Orthodox seeds can be dried to low water content at 2-6% moisture, with little effect on viability (Hong & Ellis, 1996); the recalcitrant seeds killed by desiccation beyond 20-30% cannot withstand the loss of moisture (Pritchard *et al*., 2004). The third category is intermediate between orthodox and recalcitrant seeds, can withstand partial dehydration, but they cannot be stored under conventional gene bank conditions because they are sensitive to cold and desiccation cannot increase their longevity (Ellis *et al*., 1990; Girma & Blyth, 2000). Since there are many species of trees that are threatened to extinction, it is important to document the seed biology of these species (Demel, 1993).

All these constraints could also impede the good germination of seeds and seedlings of *S. guineense*, a very nectariferous species, as many studies have demonstrated that this species is a highly nectarine bee plant that benefits from pollination by insects among which *Apis mellifera adansonii* (Hymenoptera: Apidae) is the most important (Yedomonhan, 2009; Djonwangwe *et al*., 2011). However, no data on the reproduction of *S. guineense* is available. The evaluation of the fruit resource revealed the presence of this species in the protected forest Bassila in the Guineo-Sudanian zone (Badou & Yedomonhan, 2017). This explains the choice of this natural forest for the seed harvest and to check whether its low regeneration is linked to the inability to germinate.

Materials and Methods

Study area

The experimental site considered was the University of Abomey-Calavi located in the Guinean zone, 15 km north of Cotonou. Meteorological data collected from Abomey-Calavi and covering a period of 40 years indicates that this environment experienced, like all the southern part of Benin, a subequatorial type climate with a bimodal rainfall regime with two rain seasons (April to July and October to November) intercalated by two dry seasons (August to September and December to March). The annual rainfall varies from 1100 to 1300 mm, while the average annual temperature is 25 °C with amplitude of 4 °C. Maximum temperatures are often recorded in March, while minima are generally recorded in August. Relative humidity is 96-97% at the end of the rainy season (June-July and November) and 34-36% at the end of the dry season (January-February), which is an annual average of 74%. From December to February, the harmattan a dry continental air manifests itself for a few days.

Data collecting

The storage capacity was assessed through the determination of the water content of the fruit and seed. Indeed, the germination capacity of recalcitrant seeds decreases as their moisture content decreases (Dagnechew, 2008). To this end, 300 ripe fruits, at a rate of 30 fruits per tree, were harvested and weighed immediately using an OHAUS Model CL 501 electronic scale with a range of 500 g x 0.1 g. It was noted that the seed masses were obtained by removing the pulp from half of this sample. All these seeds were subsequently dried at 60 °C in an oven for 7 days, where their mass became constant.

For the assessment of germination, two categories of fruit were considered. The first category was composed of freshly fallen and pericarp fruit intact (fig. 1A) and the second category gathered ripe fruit freshly fallen, but with truncated pericarp because previously eaten by birds (fig. 1B). These two categories of fruits were stored in the cold from the flies of fruit and mold.

Two tests of germination tests were carried out on the esplanade of the National Herbarium of Benin (HNB) of the Abomey-Calavi campus. The first trial consisted of seeds conserved for 25 days after harvest and the second was done with seeds stored for 70 days; the two storage durations being chosen at random. This made it possible to appreciate the behavior of the seeds in the face of dehydration. For this purpose, a total of 200 intact pericarp fruits and 200 truncated pericarp fruits were collected and sorted according to size.

Figure 1: Intact pericarp fruit (A) and truncated pericarp fruit (B) 25 days after storage.

On the eve of sowing, these seeds were soaked in water for 12 hours to facilitate their softening. The planting was done on the basis of one seed per low-density polyethylene nursery bags filled with sandy clay soil rich in humus resulting from the decomposition of the organic matter. The maintenance (sprinkling, weeding) of the seedlings was daily. The number of germinated seeds per day was recorded until germination was no longer present. Seedling monitoring was done for 15 weeks after germination following the recommendations of Bationo *et al.* (2001). During this period, the parameters measured on the samples of randomly selected 30 seedlings were height, collar diameter and leaf number.

Data processing

The moisture content relative to the fresh mass was then calculated by seed class by the following formula of Willan (1992):

$$100 \times (mf - ms) / mf,$$

with mf the fresh mass and ms the dry mass.

The correlation between fresh weight, dry weight and water content was determined by the Pearson correlation, which is a parametric test. The correlation coefficient (r) was calculated for this purpose.

The daily cumulative number of germinated seeds allowed to plot the germination curves for each of the 100 seed lots using the Excel Spreadsheet. The final percentage of germination (PG) is the percentage of seeds that can germinate under experimental conditions (Tang *et al.*, 2007):

$$PG = \frac{\text{total germinated seed}}{\text{total seeded seed}} \times 100$$

The Minitab 14 software allowed the analysis of variance ANOVA One-Way at the level of the germination variables for the two factors controlled in the experiment: fruit category and duration of storage.

Results

Evolution of the weight and moisture content of the ripe fruits

Case of mature fruit intact

The water content of the intact ripe fruits varied from 38 to 84.78% with an average (mean ± sd) of 72.90 ± 7.78% for all treated fruits (fig. 2A). It was positively correlated with fresh weights (r = 0.592) and showed that variation in the water content of fruits can be explained by the change in fresh weight. The best fit between water content and fresh weight was a polynomial function with 50.67% determination (fig. 2B).

The fresh weight of these fruits varied from 1.6 to 8.9 g, with an average of 4.64 ± 1.53 g. For the dry weight, it varies from 0.5 to 2.8 g with an average of 1.19 ± 0.31 g. Analysis of variance showed that the influence of drying on fruit weight varied significantly from one fruit to another (p = 0.000, r = 0.766).

The linear regression between fresh and dry weights of fruits showed that dry weights (y) can be estimated from fresh weights (x) with 58.74% success according to a linear function of equation: y = 0.1566x + 0.4607) (fig. 2C).

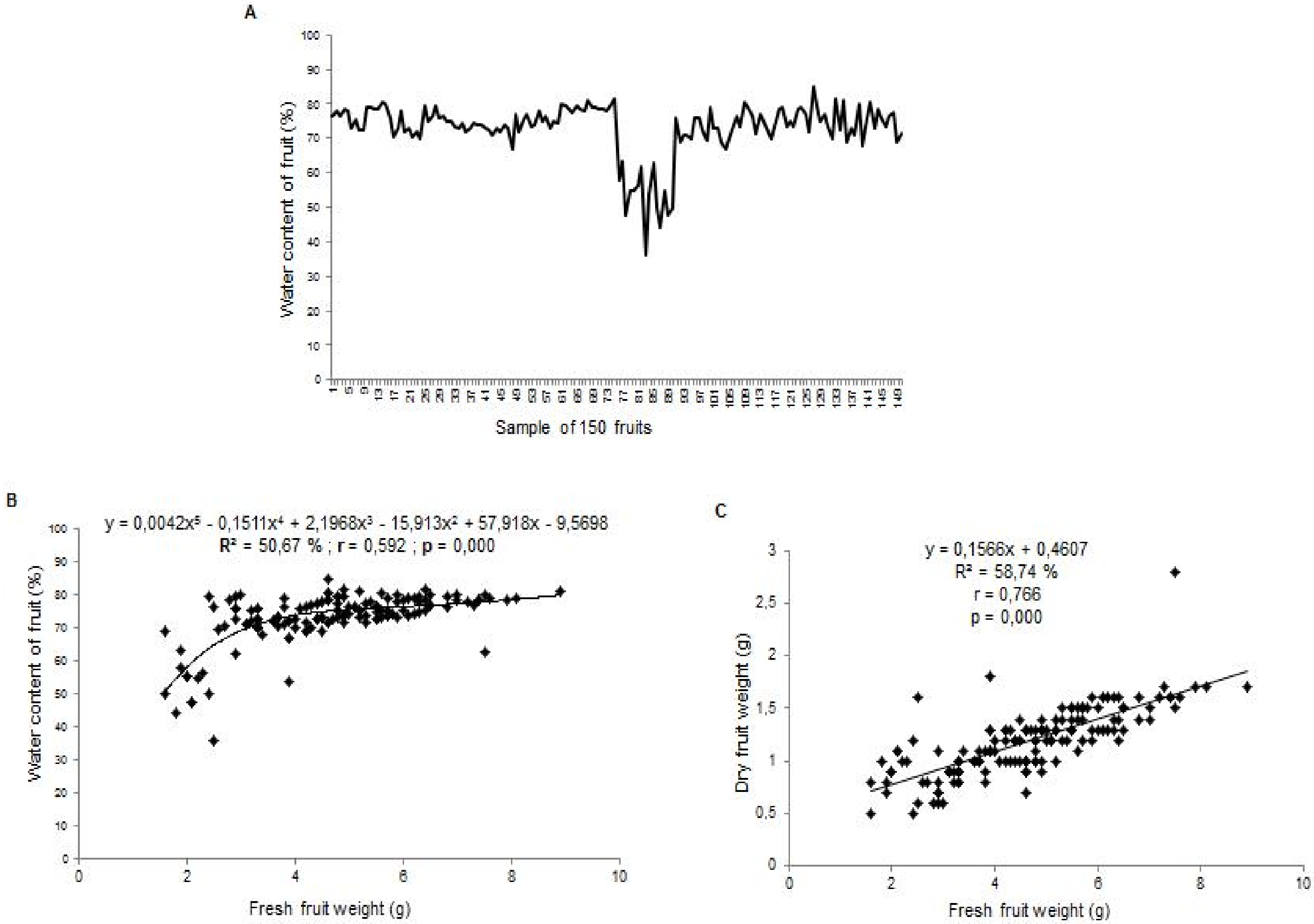

Figure 2: Variation of the moisture content and linear trend line between weights of whole fruits (R^2: coefficient of determination; r: Pearson correlation coefficient; p: probability threshold).

Case of seeds extracted from ripe fruit

The seed water content varied from 23.08 to 82.61% with an average of 55.70 ± 8.70% for all seeds treated (fig. 3A). It was weakly correlated with fresh weights (r = 0.172), suggesting that the variation in the water content of the fruits was not related to that of the fresh weights. The best fit between water content and fresh weight was a polynomial function with a low coefficient of determination (8.49%) (fig. 3B).

The fresh weight of these seeds varied from 0.7 to 5.4 g with an average of 2.10 ± 0.67 g. For dry weight, it ranged from 0.2 to 2.6 g with an average of 0.92 ± 0.32 g. Analysis of variance showed that the influence of drying on seed weight varied from seed to seed (p = 0.000, r = 0.847). The linear regression between fresh and dry weights of the seeds indicated that dry weights (y) can be estimated from fresh weights (x) with 71.71% success according to a linear function of equation: y = 0.4016x + 0.0769 (fig. 3C).

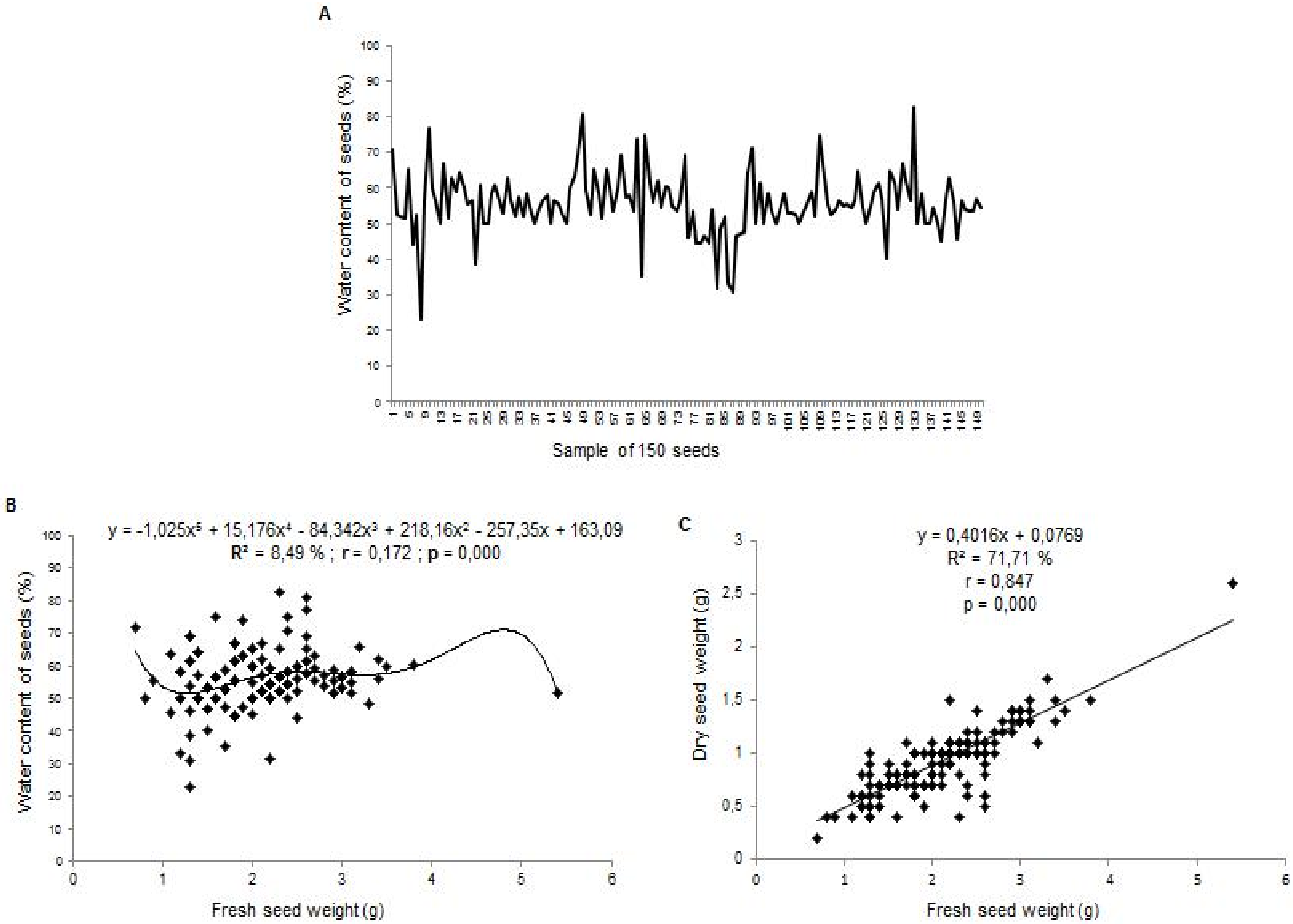

Figure 3 -- Variation of the moisture content and linear trend line between seed weights extracted from fruit (R^2: coefficient of determination; r: Pearson correlation coefficient; p: probability threshold).

Evolution of the germination rate of fruits according to the storage duration

Case of mature fruit intact

The evolution of the germination rate of the intact ripe fruit as a function of the storage duration is shown in fig. 4.

Considering the fruits preserved during 25 days before sowing, their germination started on the 36th day after sowing and ended on the 90th day. It therefore lasted for a period of three months. The evolution of the cumulative rate of sprouted fruits showed a stepped curve with uneven steps. This indicates that dormancy in these fruits was achieved by batch of fruit at irregular intervals of time. After the 90 days, no other fruit had germinated and only 42 fruits had germinated in total on the 100 seedlings, i.e. a germination capacity or a final percentage of germination (PG) of 42%.

On the other hand, in the case of intact ripe fruit that had been stored for 70 days before sowing, none of them had germinated. They lost their germinative power (fig. 4).

Case of truncated ripe fruit

The evolution of the germination rate of the truncated ripe fruits according to the storage duration was presented in fig. 4.

Considering the fruits preserved for 25 days before sowing, their germination started on the 45th day after planting and ends on the 90th day. It also lasted for a period of 3 months. The evolution of the cumulative rate of sprouted fruits showed a stepped curve with uneven steps. This indicated that dormancy in these fruits was also achieved by batch of fruit at irregular intervals of time. After 90 days, no other fruit had germinated and only 32 fruits had germinated in total on the 100 seedlings, i.e. a germination capacity or a final percentage of germination (PG) of 32%.

On the other hand, in the case of truncated ripe fruits that were kept for 70 days before sowing, none of them germinated. They all lost their germinative power (fig.4).

In fact, the percentage change in germination varied significantly from one fruit category to another (x^2 = 58,207; p = 0,000). It also depended on the number of days after sowing (p = 0.000) and the storage time (p = 0.000). However, whole fruits showed a higher germinative performance than truncated pericarp fruit by birds.

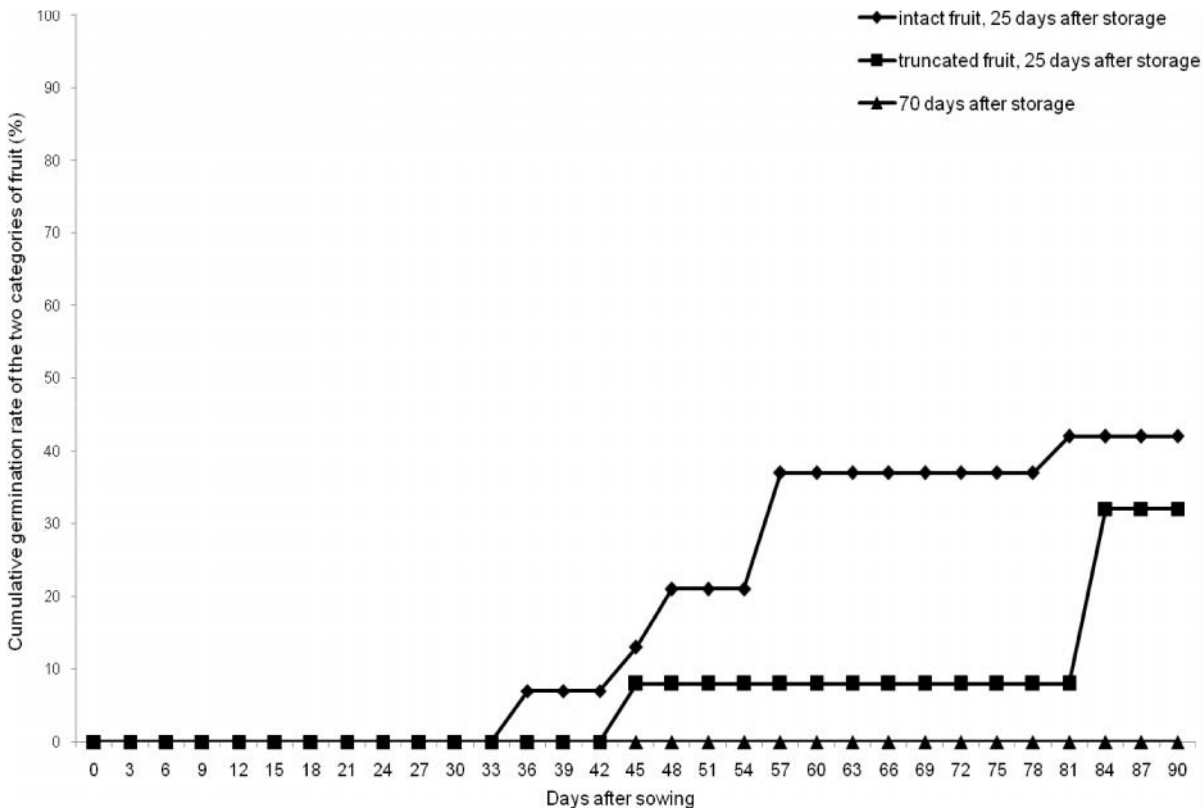

Figure 4 -- Influence of storage duration on germination of intact fruit and truncated pericarp fruit.

Monitoring of seedling survival and growth

At the end of the 15 weeks of trials, 23% of the seedlings had died from wilting. The survival rate of the seedlings was 77%. As for the growth of these seedlings, their height varied from 3 cm during the first week to 12 cm at the 15th week (table 1).

The weekly rate of stem growth was 0.60 cm, i.e. a growth of 0.086 cm per day or 2.57 cm per month. The analysis of variance showed that height growth varied significantly from one seedling to another (ddl = 29; p = 0.000) and from one week to another (ddl 14; p = 0.000).

The diameter ranged from 0.10 cm in the first week to 0.43 cm in the 15th week (table 1). The weekly growth rate was 0.22 mm, i.e. an increase of 0.03 mm per day or 0.94 mm per month. The analysis of variance showed that the diameter growth varied significantly from one seedling to another (ddl = 29; p = 0.013) and from one week to another (ddl 14; p = 0.000). The diameter of the collar was slow and not of the same magnitude as the height (p = 0.000).

For the number of leaves, it increased in pairs, progressively from 2 ± 0 to 16 ± 2 for the 15 first weeks. The growth rate was 6 sheets per month. Analysis of variance showed that leaf growth varied significantly from one seedling to another and from one week to another (p = 0.000).

Table 1 - Cumulative increase of parameters measured during the first 15 weeks of seedling growth.

Weeks	Number of leaves	Average diameter at collar (cm)	Average Height (cm)
1	02 ± 0	0,10 ± 0,0	03,03 ± 0,6
2	04 ± 2	0,10 ± 0,0	04,38 ± 0,9
3	06 ± 2	0,10 ± 0,0	05,6 ± 1,1
4	06 ± 2	0,12 ± 0,03	06,9 ± 1,4
5	08 ± 2	0,13 ± 0,03	07,4 ± 1,4
6	08 ± 2	0,14 ± 0,02	07,6 ± 1,3
7	08 ± 2	0,14 ± 0,02	08,03 ± 1,2
8	10 ± 2	0,15 ± 0,01	08,6 ± 1,3
9	10 ± 2	0,15 ± 0,01	08,9 ± 1,3
10	10 ± 2	0,15 ± 0,01	08,9 ± 1,3
11	12 ± 2	0,16 ± 0,02	09,3 ± 1,1
12	12 ± 2	0,16 ± 0,02	09,6 ± 1,1
13	14 ± 2	0,17 ± 0,02	09,9 ± 1,3
14	14 ± 2	0,36 ± 0,56	10,5 ± 1,6
15	16 ± 2	0,43 ± 0,63	11,6 ± 1,6

Discussion

Contrast between fruits moisture and their production period in the year as a cause of the low regenerations

Exploration in the protected forest of Bassila revealed the presence of regenerations of *S. guineense* ranging from 1 to 5 seedlings / ha (Badou & Yedomonhan, 2017). The disappearance of these seedlings during the dry season was linked to early mortality due to wildfires and to their trampling. However, 85% of the trees and shrubs flourished and all produced fruit with an average production estimated to1244 fruits / ha in this forest, excluding the non-productivity's assumptionof the species.

The present study also revealed that *S. guineense* subsp. *macrocarpum* yielded a mass of 2.10 ± 0.67g per fresh seed and 0.92 ± 0.32g per dry seed well above 0.27-0.42 g for a seed of *S. guineense* subsp. *guineense* which would lose its germinative power after 24 hours of storage (Eyog Matig *et al*., 2006; Maroyi, 2008; Orwa *et al*., 2009). According to the latter, the seeds of *S. guineense* would germinate easily with a high germination rate of 80-90% after 20-30 days for immediate sowing after harvest without pretreatment. In this study, the first germinations were recorded from the 36th and 45th days and then a total germination rate of 32 and 42% after only 25 days of storage. This indicates a poor response to the storage of *S. guineense* seeds, which can be explained by the seed recalcitrant nature of the species *S. guineense* (Eyog Matig *et al*., 2006; Dagnechew, 2008; Maroyi, 2008; Orwa *et al*., 2009).

Seeds of recalcitrant species have a high moisture content and lack dormancy at dispersal time (Garwood & Linghton, 1990), while low water content promotes long storage duration (Willan, 1992). According to the latter, a water content of 4 to 8% of the fresh weight favors the storage of the seeds without special precautions. This condition is contrary to that of recalcitrant seeds which cannot undergo a significant reduction in their water content without damage during their short lifetime. This study showed that the seeds of *S. guineense* had a water content of 72.90 ± 7.78% and 55.70 ± 8.70% respectively for fruits and seeds and can therefore be classified as recalcitrant seeds and extremely sensitive to desiccation. This moisture was significantly higher than that of *Afzelia africana* orthodox seeds (8.33% relative to the fresh weight) where germination was spread over a period of about one month and a germination rate still reaching 80%, 33 months after harvest (Bationo *et al*., 2001).

The relatively brittle and permeable integuments of the seeds of *S. guineense* did not protect them from desiccation (Dagnechew, 2008; N'Danikou, 2017). Our results are consistent with those obtained by Dagnechew (2008) on *S. guineense* subsp. *afromontanum*. According to the latter, the average moisture content relative to the fresh weight was 68% and 51% respectively for fruits and seeds (fruit without pulp) and the percentage of germination decreased significantly to the reduction of moisture content at 24% moisture; and even no seed germinated below 24% moisture (Pritchard *et al*., 2004; Dagnechew, 2008).

Moreover, the shift (10%) between the germination rate of the two seed categories can be explained by the fact that truncated pericarp fruits or pulpless fruits were more sensitive to dehydration than others. Similarly, the germination rate (0%) obtained after 70 days of storage can be explained by the fact that the seeded fruits had reached 0% of moisture.

According to Tompsett & Pritchard (1998), tropical recalcitrant seeds, in most cases, are limited to storage for one year. But seeds that tolerate desiccation can survive for three years. Many authors who have documented seed behavior of the Myrtaceae species have reported results displaying a high initial seed viability; this viability gradually decreases during desiccation (Baxter *et al*., 2004; Wang *et al*., 2004; Dagnechew, 2008). Other authors have pointed out that sensitivity to desiccation of seeds is potentially a high risk for plant germination and regeneration (Hong & Ellis, 1996; Barbedo & Bilia, 1998; Pritchard *et al*., 2004).

Sensitivity or affinity of *S. guineense* fruit to infectious agents and birds

The proliferation of fungi can also explain seed death; most authors have reported this problem in germination experiments of recalcitrant seeds of species such as *S. guineense*. Omondi (2004) and Dagnechew (2008) reported the proliferation of fungi in the germination experiment of *S. guineense*. Anguelova-Merhar *et al*. (2003) highlighted the role of microflora and in particular fungal species as an important factor in determining seed life.

According to the inventories of Vayssières *et al*. (2010) in Benin, *S. guineense* is among 20 local fruit species with fruiting periods from March to October in the Guineo-Sudanian zone in Benin. The pulp of the large majority of these fruits is exploited by the larvae of several species of *Ceratitis* and by a formidable invasive species that ranged in sub-Saharan Africa, *Bactrocera invadens* (Diptera, Tephritidae).

Investigations on the various hosts of fruit flies revealed the infestation of the majority of local fruits including those of *S. guineense* by these frugivorous pests. The two polyphagous species of Tephritidae, *Ceratitis cosyra* and *Bactrocera invadens* are the most abundant and those which generate the largest losses of production (Vayssières *et al*., 2010).

In the case of *S. guineense* predators, fruits are eaten by birds, monkeys and wild animals (Burkill, 1997; Dagnachew, 2008). According to Tang *et al*. (2012), there are two species of Frugivorous Bat (*Cnopterus sphinx* and *Rousettus leschenaulti*) which are seed dispersers of *Syzygium oblatum* (Myrtaceae). These bats cause more than half of the fruit produced by the tree (65%) to fall to the ground. Apart from the fruits collected, they are also able to disperse the seeds up to 73 m of the seeds, thus facilitating the regeneration of *S. oblatum* (Tang *et al*., 2012). In our study area, birds consumed only the pulp. These birds can be assumed as *S. guineense* seed dispersers.

Conclusion

The present study made it possible to understand that the seeds of the species germinate both in experimentation and in the natural environment.

The dehydration capacity and the rapid loss of germinative capacity of the generative material of *S. guineense* was a major constraint to its regeneration.

Immediate sowing is therefore advantageous for *S. guineense*. In view of current knowledge of the regeneration of *S. guineense*, beekeepers, foresters and riparian populations must be actively involved in the conservation of forests and savannas at *S. guineense* through the development of nurseries for the introduction of this species very nectariferous in the strategies of reforestation for apiculture purposes.

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