International Journal of Innovation Scientific Research and Review
Vol. 03, Issue, 10, pp.1889-1893, October, 2021
Available online at http://www.journalijisr.com
SJIF Impact Factor 4.95

ISSN: 2582-6131

Research Article

FIRST ASSESSMENT OF CENTRAL AFRICAN FOREST DUIKER AND ELEPHANT DIET USING FECES EXTRACTED SEEDGERMINATION

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Received 23th August 2021; Accepted 26th September 2021; Published online 31th October 2021

ABSTRACT

Central African duikers and elephants play a major role in seed dissemination, thus regenerating and structuring the forest. However, this forest mammal ecology is still poorly known, especially their diet. One possible explanation might be to gather field data and to experiment in a limited constrained environment. So, this study aimed to use a simple and efficient technique, seed germination, to determine central African forest duiker and elephant diet. A total of 43 fecal samples (34 duikers and 9 elephants) were collected in Lopé national park, central Gabon, and germinated into 96 pots containing either sterilized soil or cotton discs. Of the 96 germinating pots, 30 succeeded (31%) and 66 failed (69%); the highest germination success rate was recorded in soil (44%) and in elephant extracted seeds (33%) respectively. Furthermore, seven plant taxa were identified out of the germination experiment: Cesalpiniaceae, Lamiaceae, Loganiaceae, Melastomataceae, Rubiacaee, and two unidentified taxa. Of these, most germination success (23/30; 76,67%) were from the Rubiaceae family and more specifically from the savanna plant species *Sarcocephaluslatifolius* (Smith) Bruce(also known as *Nauclea latifolia*). Results of the present study proved to be useful as a first step in determining Central African duiker and elephant diet. However, these results should be further complemented with other techniques such as DNA metabarcoding or isotope analyses.

Keywords: Seed germination, forest duikers, forest elephant, diet, Lopé national park.

INTRODUCTION

The tropical forest of Central Africa is one of the richest regions of the world in terms of biodiversity (Malhi *et al.,* 2013). This is largely due to the role that ecosystem engineers, such as forest duikers and elephants, play structuring and regenerating the forest (Feer 1989b; Wilkie *et al.,* 2011; Omeja *et al.,* 2014). Indeed, these medium size and large mammals represent an important component of the forest community, most of them being herbivores and frugivores. For instance, forest duikers and elephants disseminate seeds for the benefit of numerous plants (Dubost 1984; Mcnaughton & Georgiadis 1986; Feer 1989a, 1995; Terborgh *et al.,* 2016). Moreover, for some species of plants, seeds going through the digestive tract of these animals is required for their germination (Wenninger & Shipley 2000; White & Edwards 2000; Brugiere *et al.,* 2002). However, forest duikers and elephants face several threats including habitat fragmentation and loss (Laurance *et al.,* 2006; Malhi *et al.,* 2013), hunting (Wilkie & Carpenter 1999; Newing 2001), increasing human population (Wilkie & Carpenter 1999), poverty (Nielsen 2006), emergence of infectious and epidemic diseases (Lahm *et al.,* 2007) and civil wars (Kanyamibwa 1998). Several species of forest duikers are threatened (i.e., *C. sylvicultor*, *C. leucogaster*, *C. dorsalis*) and the African forest elephant (*Loxodonta cyclotis*) is now assessed as "Critically Endangered" (Gobush *et al.,* 2021; Zhongming *et al.,* 2021), thus highlighting the importance of putting in place a conservation and sustainable management plan. Though, forest duikers and elephant are still poorly known and studied, especially in terms of their diet (Short 1981; Dubost 1984; White *et al.,* 1993; Feer 1995; Debruyne 2005). This situation is even worse in developing countries where high throughout DNA sequencing facilities are still very limited (Herbert 2001; Wynberg 2002; Swartz *et al.,* 2008). Thus, the aim of this study was to successfully germinate seeds extracted from forest duiker and elephant feces. This information is critically important because it will not only uncover forest duiker and elephant diets, but also reveal the set of plants these animals disperse. Furthermore, outcome of this study will provide useful data to decision makers for appropriate and targeted conservation action plans within forest duiker and elephant distribution range and preferred habitat.

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MATERIAL AND METHODS

Study area and feces collection

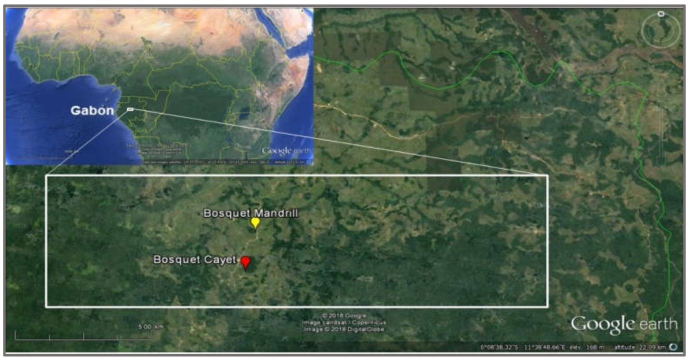

Figure 1: Sampling locations

Data collection was carried out on July 19-29, 2014 in Lopé National Park (LNP), Gabon, within the forest-savanna mosaic matrix located on its northeastern part (White & Abernethy 1996; Ango 2008) (Figure 1). This protected area covers about 4913 km² and is characterized by a relatively lesser rainy climate; annual rainfall being about 1502 mm, while the rest of Gabon is at about 2000 mm (Moumaneix & Nkombe 2017). Sampling was done along six line transects of 300 meters each in two forest fragments (Cayet and Mandrill) and in continues forest (Figure 1). Duiker and elephant feces of 48 hours to 2 months old were collected and stored into 14 ml Falcon tubes and 0.5-liter zip lock plastic bags, respectively. All Falcon tubes and zip lock plastic bag contained silica bids to preserve feces from moisture and to guaranty higher DNA amplification rate in downstream analyses (Soto-Calderon *et al.,* 2009). Sampled feces were stored at room temperature for two weeks prior to the germination experiment in the laboratory.

Seed extraction from feces and germination

First, sprouts that had germinated from elephant's feces before the germination experiment even started were removed so that they wouldn't be confused with those of the controlled experiment. Then, elephant feces were dried at room temperature for an additional seven days. Once dried, seeds extracted from elephant feces were classified according to their size, using one- and three-millimeters diameter hole sieves consecutively: large seeds (>3 mm), medium size seeds (1-3 mm), and small seeds (<1 mm)(Walsh & Lord 1996). Duiker's feces were put in tap water for two weeks (Tiwari *et al.,* 2018) to soften them because they had become very hard and dry after two weeks of storage in silica gel. When softened, duiker's feces were crumbled using sterilized wooden toothpicks. Plastic bottles of 1,5 liter were cut at 10-15 cm height to make germination pots (Figures 2a, b, and 3). These pots were covered with aluminum foil to make them opaque to any source of light which can have a negative effect on root growth (Ohno & Fujiwara 1967). In addition, tiny holes were made at the bottom of each pot, allowing the overflow of water to be evacuated and to aerate the germinating substrate (Mile to, 2009). Two locally available substrate types were used to start the germination process in the laboratory: sterilized soil and cotton discs (Figures 2a and b). The soil was collected from a sand quarry in Franceville, Gabon, and heat sterilized at 105° Celsius for two hours(Mokossesse *et al.,* 2009). Then, two duplicates for duikers and 2-7 duplicates for elephants, depending on the number of extracted seeds per sample, were distributed in a total of 96 germinating pots as shown in Table 1.

Table1. Number of germinating pots (samples) used with sterilized soil, cotton discs.

	Soil	Cotton	Total
Elephants	16	12	28
Duikers	34	34	68
Total	50	46	96

All germinating pots were watered with tap water at one to two days intervals (Plantes-et-jardins 2014). Additionally, 70 mg of NPK fertilizer 10/10/20 diluted in 10 liters of tap water was used every 15 days to accelerate growth of sprouting (Plantes-et-jardins 2014). Then, 0.5 liter of the previous solution was diluted in one liter of water and finally served for watering (2.33 mg/l NKP final concentration). After three months of in vitro substrate culture, the young plants were placed outside on ground soil to continue the growth process. After six months of development, seedlings were identified using characters such as vegetative organs (stem and leaf), presence of stipules, hairs, exudate, and aromatic odors (Tomlinson 1987). Accepted name of each species was carried out using the online plant database "The-Plant-List" (The-Plant-List 2013). Finally, the Chi-square test was implemented to assess the relationship between the outcome of the germination experiment (failure or success) and the type of substrate used.

RESULTS

Germination experiment

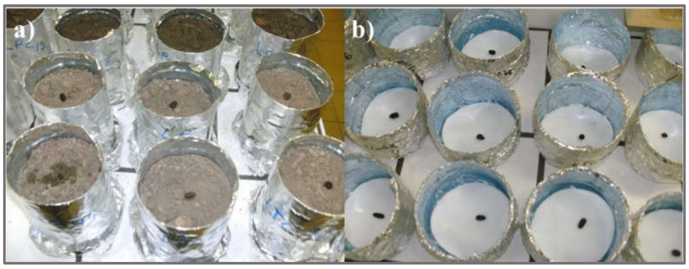

Figure 2: Germination pots

Table 2. Number (and percentage) of pots with germination success and failure out of sterilized soil and cotton.

	Soil	Cotton	Total
Success	22/50 (44%)	8/46 (17%)	30 (31%)
Failure	28/50 (56%)	38/46 (83%)	66 (69%)

Of the 96 germinating pots, 30 succeeded (31%) and 66 failed (69%) (Figure 2 and Table 2). The highest germination success rate was recorded in soil (44%), while the highest germination failure rate was in cotton (83%) (Table 2). There was a significant relationship between the outcome of the germination experiment (failure or success) and the type of substrate used (χ^2 = 6.70;p-value= 0.01).

Table 3. Number of success and failure (and percentage) of germination from elephant and duiker feces.

	Elephant	Duiker	Total
Success	10/28 (33%)	20/68 (29%)	30/96 (31%)
Failure	18/28 (67%)	48/68 (71%)	66/96 (69%)

Comparing animal taxa, the highest germination success rate was recorded in elephant (33%), while the highest germination failure rate was in duiker (71%) (Table 3). There was not any significant relationship between the outcome of the germination experiment (failure or success) and the origin of germinated seeds (χ^2 = 0.13; p-value = 0.71).

Plant identification

Figure 3: Grown plants

Of the30 pots that successfully germinated, a total of seven (7) plant taxa were identified: four (4) known families and two unidentified taxa

(Figure 3). From these taxa, six (6) germinated from elephant feces and three (3) from duiker feces. Two (2) families (Rubiaceae and unknown family 2) germinated from both animal feces. Three (3) families (Cesalpiniaceae, Lamiaceae, Loganiaceae, Unknown Taxa 1) were specific to elephants, whereas only one (Melastomataceae) was specific to duikers (Table 4).

Table 4. Number of germinations per plant family from elephant and duiker extracted seeds.

Families	Elephants	Duikers	Total
Cesalpiniaceae	1	0	1
Lamiaceae	2	0	2
Loganiaceae	1	0	1
Melastomataceae	0	1	1
Rubiaceae	3	19	22
Unknown Taxa 1	1	0	1
Unknown Taxa 2	1	1	2

However, most germinated plants were from the Rubiaceae family (N = 22/30) and more specifically from *Sarcocephaluslatifolius* (Smith) Bruce, which is a savanna plant species (Table 4).

DISCUSSION

The present study showed a higher germination success rate in the soil than with cotton, and in elephant than in duiker. Indeed, it is known that soil properties can affect seed germination and plant growth (Gallandt *et al.,* 1999). Although external environmental factors such as light, humidity, temperature, water, nutrients, and even pathogens are known to influence soil quality and seed germination (Grouzis *et al.,* 1976; Leblanc *et al.,* 1998; Gallandt *et al.,* 1999), these were kept constant during the experiment. Comparing both taxa, the role of elephant as seed disperser is already well documented for several plant taxa (Gautier-Hion *et al.,* 1985; Feer 1995; Blake & Inkamba-Nkulu 2004; Born *et al.,* 2008; Blake *et al.,* 2009; Ndiade-Bourobou *et al.,* 2010; Campos-Arceiz & Blake 2011; Beaune *et al.,* 2013; Bunney *et al.,* 2017). Other factors, such as the season of collection, seed storage, burial depth and treatments could have also influenced seed germination (Tozo *et al.,* 2004). For instance, the relative proportion and composition of duiker diet varies with the season (Feer 1989a; Hofmann & Roth 2003), thus affecting fecal seed content and thereafter seed germination success. Moreover, not all plant species will be affected the same way by different soil types (Delgado & Gómez 2016), because they have their own properties that depend on the nature of their mineral and organic matter, their relative amounts, and the way these minerals and organic matter are associated (Brady & Weil 2000). Thus, certain plant taxa may had been favored by the tested soil type in this study, while others could not germinate at all. The lack of significant relationship between the outcome of the germination experiment (failure or success) and the animal (forest duikers or elephant) making the feces from which was extracted the seed, could be due to the low sample size. Moreover, the diameter of ingested seeds, their teguments or the internal digestive physicochemical environment can influence seed germination. In this sense, it has already been recorded successful seed germination after transitioning through elephant digestive tract of tropical plants such as *Balanites wilsoniana, Chrysophyllumlacourtianum, Myrianthusarboreus, Lagenaria breviflora, Dubosciaviridiflora, Celtis mildbraedii, Tetrapleura tetraptera, Treculiaafricana, Costusafer, Cissus sp., Cucumis sp.*(Chapman *et al.,* 1992; Theuerkauf *et al.,* 2000; Cochrane 2003; Beirne *et al.,* 2019). The identification of successfully grown plants revealed that most were from the Rubiaceae family, and more precisely from *Sarcocephaluslatifolius*. This plant is a savanna species that occurs in the northeastern part of LPN and it is easily recognizable by its large thick opposite leaves with red petioles (White & Abernethy 1996). S. latifoliushas already been cited as part of the elephant's diet (White & Abernethy 1996). Regarding duikers, it has been shown that *Philantombamaxwellii* consume *S.latifolius*in western Africa (Hofmann & Roth 2003; Skrzynski 2006). The reason of such a higher frequency in both elephant and duiker feces might be that *S.latifolius* produces big red-brown fruits that contain thousands of tiny seeds (White & Abernethy 1996), increasing their probability of passing through their digestive tract and end up in feces.

CONCLUSION

Results of this study suggest that forest elephants and duikers of LNP feed on the savanna plant species *S.latifolius*. This would suggest that these forest dweller species may wonder in the savanna, especially in the dry season, when their preferred foods are less available in the forest. Thus, seed germination appears as a useful tool for diet preliminary studies of frugivorous targeted taxa. Advantages of this method include its simplicity, reproducibility, and low cost. However, the method used herein has obviously several limitations, including the impossibility to recover completely digested seeds, leaves, and others food items known to be part of elephant and duiker diets. Furthermore, results of the present study could be complemented with additional analyses of regurgitated food (i.e., for duikers) and molecular techniques such as metabarcoding (Pompanon *et al.,* 2012; Quéméré *et al.,* 2013) or isotope analyses (Schoeninger *et al.,* 1999; Bocherens 2009; Hoffecker 2009; Richards 2009).

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