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Assessing the role of a mammalian frugivorous species on seed germination potential depends on study design: A case study using wild samango monkeys

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Author contributions: SS, RH, LS, SD and NK conceived and designed the experiments. SS performed the experiments. SS and BL collaborated in seed nursery design, construction, and seed identification; SS, RH, LS and NK designed analyses; SS analysed the data and wrote the manuscript; RH, LS; SD, BL and NK revised manuscript drafts.

1 **ABSTRACT**

2

3 Frugivory and seed dispersal contribute to the maintenance and regeneration of plant communities through
4 transportation of seeds and enhancing germination through seed processing mechanisms. The effects of
5 mammalian frugivore seed processing mechanisms on seed germination are generally well studied and the
6 potential benefits include disinhibition (pulp removal), scarification (gut passage) and fertilisation (from faecal
7 matrix). Nevertheless, our review found that there is bias in the comparative treatments included in seed dispersal
8 studies through exclusion of entire fruit control groups and the fertiliser effect. In this study, we aimed to address
9 such bias by using ecologically relevant experiments to investigate the influence of seed processing mechanisms
10 on germination probability and latency of selected locally abundant fleshy-fruited plant species, common in the
11 diet of samango monkeys (*Cercopithecus albogularis schwarzi*), and who are seed-spitters (disinhibition) and
12 seed-swallowers (gut-passage and fertiliser effects). We designed experiments to isolate the cumulative effects of
13 seed processing mechanisms and tested the effects of five treatments and one control treatment (entire fruit). We
14 further assessed if exclusion of ecologically relevant seed treatments or relevant controls would affect our
15 interpretation of the impact of the disperser on seed germination. Comparing gut passage and disinhibition
16 indicated negative effects, whereas comparing gut passage and entire fruit controls indicated neutral effects in one
17 species. Compared with gut passage alone, the fertiliser effects indicated positive or neutral benefits on
18 germination probability. Our study demonstrated that the impacts of mammalian frugivores on germination may
19 be under- or over-estimated in ecological literature where relevant treatments and meaningful controls are
20 excluded.

21

22 **KEYWORDS**

23 Control; Entire Fruit; Gut passage; Frugivore; Dispersal; Seed handling; Scarification

24

25

26

27

28 1. INTRODUCTION

29 Frugivory and seed dispersal form an important mutualistic relationship (Farwig and Berens 2012). Across many
30 habitats, plants rely on frugivores to disperse seeds away from the parent (Janzen 1970; Connell 1971) and
31 enhance germination and seedling establishment (Terborgh et al. 2002; Traveset et al. 2007). Germination
32 experiments have been vital in understanding the role that vertebrate dispersers play in seed dispersal effectiveness
33 (Schupp et al. 2010) and thus the contribution of frugivory to the maintenance and regeneration of plant
34 communities (Janzen 1970; Howe and Smallwood 1982; Traveset et al. 2007). The effectiveness of a disperser
35 depends on the quality of dispersal (the probability of seed germination and survival to reproduction) and quantity
36 of seeds dispersed (Schupp 1993). The quality of dispersal refers to any potential enhancement of germination
37 through seed processing mechanisms, such as passage through the gut of the disperser (Schupp 1993; Schupp et
38 al. 2010. Traveset 1998), which are extrinsic to deposition into microsites favourable for germination and survival.

39
40 The effects of frugivore seed processing mechanisms on seed germination are well studied (Traveset 1998;
41 Traveset et al. 2007; Fuzessy et al. 2016) and directly affect seed germination in three ways (Traveset and Verdú
42 2002). Firstly, removal of fruit pulp (*the disinhibition effect*) releases seeds from chemicals that inhibit
43 germination and decrease the risk of fungal-mediated mortality (Traveset and Verdú 2002). Secondly, passage
44 through the gut or fine oral processing can enhance germination by breaking seed dormancy through mechanical
45 and chemical scarification of the seed coat (*the scarification effect*) which, in addition to releasing the seed from
46 inhibiting chemicals through pulp removal, may increase its permeability to water and gases (Traveset and Verdú
47 2002; Baskin and Baskin 2014). Thirdly, in mammals the faecal matrix can enhance seed germination through a
48 fertilising effect, or can have a lethal effect by facilitating the growth of fungus and/or bacteria (*the fertiliser*
49 *effect*) (Traveset and Verdú 2002; Traveset et al. 2007). The majority of studies focus on the effect of gut passage,
50 reporting an increase in the number of seeds germinating and decrease in latency to germinate (time from seed
51 deposition to germination) (e.g. Petre et al. 2015; Muñoz-Gallego et al. 2019). However, despite previous calls
52 for ecologically valid comparative experiments to ascertain the effect of processing mechanisms and the
53 importance of entire fruits as controls (e.g. Samuels and Levy 2005), there is still a prominent bias in the
54 comparative treatments included in seed dispersal studies (Fuzessy et al. 2016).

55

56 It has been 14 years since Samuels and Levey (2005) posed the question “*Do germination experiments answer*
57 *the questions they ask?*”, highlighting the importance of choosing appropriate treatments to answer questions

58 about the effectiveness of gut passage on seed dispersal. At that time 77% of studies used the comparison between
59 germination of gut-passed and manually extracted seeds in their study design (Samuels and Levy 2005). We
60 therefore conducted a review of the literature since 2005, restricting our search to mammals only, to assess
61 whether studies are now appropriately designed to answer such questions. We used the following search terms in
62 a Web of Science topic search (which includes title, abstract and keywords) for the period January 2005 to May
63 2019: GERMINATION, SEED, EXPERIMENT OR DISPERS*, "GUT PASSAGE" OR "SEED HANDLING"
64 OR SPIT*. Whilst noting that the review was not exhaustive, the search generated 212 papers in total, 69 of which
65 investigated the likelihood of seed germination following spitting by, or passage through the gut of, mammal seed
66 dispersers (Supplementary Information Table A).

67

68 Of these 69 studies, 62% used the disinhibition effect, rather than entire fruit, as the control treatment. Of the 59
69 studies investigating dispersal of fleshy-fruit seeds, 76% omitted entire fruit controls. Of the 66 studies
70 investigating dispersal via gut passage 76% excluded the fertiliser effect of faecal matrix. Furthermore, only 9%
71 of studies included manual, chemical and/or mechanical scarification of de-pulped seeds along-side gut-passed
72 seeds, which would isolate the effect of scarification processes. Without differentiating each fundamentally
73 different mechanism, the absolute effect of frugivores on seed germination is confounded (Samuels and Levey
74 2005; Robertson et al. 2006; Baskin and Baskin 2014) and these studies are weakened in their conclusions.
75 Therefore, it is important to consider all processes and include entire fruits as a control group to estimate describe
76 the role of frugivores in seed dispersal.

77

78 An additional issue is that many experiments are conducted *ex-situ* in laboratories (e.g. Kankam and Oduro 2012;
79 Lessa et al. 2013), growth chambers and incubators (e.g. Carvalho-Ricardo et al., 2014; Maldonado et al., 2018),
80 while few studies (e.g. Chen et al., 2016; Sánchez de la Vega and Godínez-Alvarez, 2010) conduct germination
81 experiments in the field where natural environmental conditions fluctuate. Experiments conducted in controlled
82 conditions often report that the effect of gut passage on seed germination is consistently higher than controls,
83 whilst in field experiments, studies most often report no significant difference (Fuzessy et al. 2016), highlighting
84 the importance of well-designed and controlled *in situ* studies.

85

86 Therefore, the aim of our study was two-fold. Firstly, we investigated the effect of seed processing mechanisms
87 by a mammalian disperser on seed germination using all ecologically relevant seed treatments and entire fruit

88 controls. Secondly, we assessed if a biased study design, excluding one or more of those treatments, would under-
89 or over-estimate such a role. Specifically, how would our interpretation of the role of our frugivore in seed
90 dispersal change if we excluded entire fruits as control groups and/or the fertiliser effect from our experiments,
91 as is often the case in the literature. We predicted that, compared with entire fruit controls, seeds processed by a
92 mammalian frugivore would demonstrate increased germination potential. We predicted that by excluding entire
93 fruit controls and the fertiliser effect from our analyses, we would underestimate the role samango monkeys have
94 in enhancing seed germination potential as we would be excluding a baseline from which to measure the
95 magnitude of the effect and ecologically relevant seed processing mechanisms, namely the disinhibition effect
96 and the faecal matrix.

97

98 In this study we focussed on a species of guenon monkey, the samango monkey (*Cercopithecus albogularis*
99 *schwarzi*), as they make an ideal model species for seed dispersal studies for several reasons. Firstly, primates can
100 consume larger fruits and seeds than most birds (Chapman and Onderdonk 1998; Balcomb and Chapman 2003),
101 process fruit in different ways, and can be classified as seed-swallowers, seed-spitters and seed-destroyers (Corlett
102 and Lucas 1990). The majority of primates species are seed swallowers (Lambert 1999; Clark et al. 2002; Dominy
103 and Duncan 2005), however guenons are unique in that they display multiple seed-processing mechanisms. They
104 are generally seed-swallowers of seeds smaller than 4 mm (on longest axis), seed-spitters of seeds larger than 4
105 mm (Lambert 1999; Gross-Camp and Kaplin 2011; Linden et al. 2015) and seed destroyers as they can crush
106 seeds during oral processing of fruits and often consume unripe fruits (Wrangham et al. 1998; Lambert 1999).
107 Mechanical and chemical scarification of seeds during oral processing may have similar effects to the scarification
108 effects during gut passage, though to a lesser degree (Anzures-Dadda et al. 2016), or may have only a disinhibitory
109 effect through pulp removal.

110

111 Secondly, guenons have a broad distribution from Ethiopia to South Africa (Lawes 1990; Grubb 2003) and are
112 highly frugivorous, with more than 50% of their diet consisting of fruit (Lawes 1991; Chapman et al. 2002). In
113 South Africa, compared with other South African primates, samango monkeys spend most of their time in and are
114 highly dependent on, high-canopy evergreen forest (Linden et al. 2015). Samango monkeys are the only large-
115 bodied arboreal species that feed on medium to large sized fruits in the canopy layer of South Africa's evergreen
116 forests (Coleman and Hill 2014; Linden et al. 2015, 2016). As such, the loss of samango monkeys could have
117 negative cascading effects on the forests in which they reside.

118

119 We designed our germination experiments to isolate the effects of processing mechanisms on germination success.

120 We selected five locally abundant fleshy-fruited plant species that represented different seed processing

121 mechanisms and were common in the diet of samango monkeys (Coleman 2013; Linden et al. 2015). The five

122 species were three *Ficus* spp. which we assessed at genus level and two fleshy-fruited tree species, *Searsia*

123 *chirindensis* (red currant) and *Syzygium cordatum* (water berry), which we assessed at species level. We assessed

124 the effects of five treatments that represented biological processes and one control treatment (entire fruit). The

125 treatments were the disinhibition effect (simulating spitting and isolating pulp removal), scarification effect

126 (simulating gut passage and isolating the mechanical effects of gut passage), gut passage effect (collected from

127 faeces and isolating the chemical effect of gut passage) and the fertiliser effect (sown in faeces and isolating the

128 effect of the faecal matrix). We also tested the added effect of seed desiccation following the disinhibition effect

129 for one species, to simulate dispersal into dry habitat.

130

131 **2. METHODOLOGY**

132 **2.1 Field Experiments**

133 **2.1.1 Study Area**

134 We conducted germination trials between January and May 2018 at the Lajuma Research Centre (29°26'E,

135 23°01'S) in the far western Soutpansberg mountain range of South Africa's Limpopo Province. The Soutpansberg

136 has an altitudinal range between 1150 m to 1750 m and local climate conditions are mesothermal with distinct

137 cool dry winter (April to September) and warm wet summer (October to March) seasons (Munyati and Kabanda

138 2009). Vegetation at the study site is characterised by a complex mosaic of vegetation types including Limpopo

139 Mistbelt Forest, Soutpansberg Moist Mountain Thickets and Soutpansberg Mountain Bushveld (Maltitz et al.

140 2003; Mostert et al. 2008). The south-facing ridges of the mountain range receive orographic and increased

141 localised rainfall (Mostert 2006) which support high-canopy evergreen forests at their base (Linden et al. 2015).

142 Away from the ridges, the evergreen forest transitions into semi-deciduous woodland, thicket, grassland and

143 intersecting riverine forest (Maltitz et al. 2003; Mucina and Rutherford 2006).

144

145 **2.1.2 Study Species**

146 In this study we followed two groups of well habituated samango monkeys, "Barn Group", comprising

147 approximately 40 individuals and "House Group", comprising approximately 60-70 individuals (Emerson et al.

148 2011; Coleman and Hill 2014; Nowak et al. 2014), between December 2016 and May 2017. Both groups
149 comprised one resident male, multiple females, and their sub-adult and infant offspring. Between March and May,
150 four to six bachelor males joined both groups intermittently for the mating season. The monkeys have been studied
151 since 2004 (Ian Gaigher, pers. comm.) and we were able to follow them at a minimum distance of 5 m, using
152 binoculars when they were high in the canopy.

153

154 For germination experiments, we selected fruiting tree species based on samango monkey feeding preferences in
155 the preceding years (2015 – 2017 inclusive). We calculated the annual proportion of fruit species in the diet using
156 data collected by the Primate and Predator Project, as described by Coleman (2013), subsequently selecting the
157 ten most consumed fruit species, in terms of proportion of time spent feeding. We also took into account the ways
158 in which the monkeys disperse the seeds, as well as the fruiting season of the trees, ensuring we were able to
159 collect sufficient quantities of monkey-dispersed seeds and fresh ripe fruits for controls and manipulated
160 treatments. Based on these criteria, we selected three *Ficus* species, *F. burkei* (common wild fig), *F. craterostoma*
161 (forest fig), *F. sur* (broom-cluster fig), and two other species, *Searsia chirindensis* (red currant, formerly *Rhus*)
162 and *Syzygium cordatum* (water berry) (Table 1). We could only identify *Ficus* spp. seeds recovered from faecal
163 samples to genus level due to the high similarity and very small (< 2 mm) seed size. However, we are confident
164 that only these three species of *Ficus* appeared in faeces, as there were no other *Ficus* species present in the area.
165 Because we were not able to distinguish between *Ficus* spp. seeds we pooled all *Ficus* spp. for subsequent
166 analyses. All three species were monoecious (hermaphrodite) (van Noort and Rasplus 2020) and we assigned
167 seeds and entire fruits randomly to each treatment. We observed the primary method of dispersal by samango
168 monkeys for *Ficus* spp. and *S. chirindensis* seeds to be via ingestion and defecation. Monkeys dropped partially
169 consumed fruit under the parent tree and, to a lesser degree, spat out seeds either under the parent tree, or away
170 from it following storage in cheek pouches (Linden et al., 2015). Monkeys spat *S. cordatum* seeds under the parent
171 tree as they consumed the fruit, and occasionally away from the parent tree following storage in cheek pouches.
172 Seeds of *Ficus* spp. and *S. chirindensis* are orthodox and can withstand desiccation whilst *S. cordatum* seeds are
173 recalcitrant and are sensitive to desiccation (Roberts 1973; Royal Botanic Gardens Kew 2017).

Species ^a	Plant and Dispersal Traits		Mean Dimensions (mm ± SD)			Published Seed Data			
<i>Scientific Name</i> (Family) Common Name Local Venda Name	Habitat ^b	Typical Fruiting Period ^b	Samango Dispersal (% Diet) ^c	Fruit H x W	Seed H x W	\bar{x} no. seeds	Desiccation Tolerance	Germination	
								Time (days)	Proportion (%)
<i>Ficus burkei</i> (Miq.) Miq. (Moraceae) Common wild fig Muumo	woodland / wooded grassland	year-round	Ingest / Spat (9.3%)	12.1 x 10.6 (± 1.7 x 1.8)		122 (± 44)			
<i>Ficus craterostoma</i> Warb. ex Mildbr. & Burret (Moraceae) Forest fig Tshikululu	evergreen forest (strangler fig)	Aug - Dec (variable)	Ingest / Spat (8.7%)	14.1 x 12.1 (± 0.9 x 1.1)	genus \bar{x} 1.3 x 0.8 (± 0.1 x 0.1)	180 (± 59)	Orthodox ^d	42 – 70 ^d	64 – 100 ^d
<i>Ficus sur</i> Forssk. (Moraceae) Broom cluster fig Muhuyu	forest / riverine fringes / open woodland	Sep - Mar (variable)	Ingest / Spat (9.4%)	29.4 x 33.2 (± 4.2 x 6.1)		795 (± 319)			
<i>Searsia chirindensis</i> ⁱ (Baker f.) Moffett (Anacardiaceae) Red currant Muvhadela-phanga	open woodland / mountain scrub / forest	Nov - Feb	Ingest / Spat (7.1%)	3.8 x 4.1 (± 0.4 x 0.5)	3.4 x 4 (± 0.5 x 0.4)	1	Orthodox ^e	35 – 56 ^f	not available
<i>Syzygium cordatum</i> Hochst. Ex C.Krauss (Myrtaceae) Water berry Mutu	occurs near water in a variety of habitats	Dec - April	Spat (4.3%)	13.1 x 9.1 (± 1.3 x 1.1)	8.9 x 6.6 (± 1.6 x 1.1)	1	Recalcitrant ^g	18 – 40 ^h	90 - 99 ^h

174 ^a *Ficus* spp. seeds and entire fruit controls were selected randomly from these three species as we were unable to identify seeds retrieved from faecal samples to species

175 ^b Coates-Palgrave 2002

176 ^c 2015 – 2017, based on data collected as described in Coleman 2013

177 ^d Royal Botanic Gardens Kew 2017 Data only available for *Ficus sur*; 100% of 52 known taxa of genus *Ficus* are Orthodox.

178 ^e Royal Botanic Gardens Kew 2017 Data not available for *S. chirindensis*; 100% of 8 other *Searsia* taxa are Orthodox.

179 ^g Royal Botanic Gardens Kew 2017

180 ^h Wilson and Downs 2012 Seeds soaked 24 h prior to sowing

181 ⁱ Formerly *Rhus chirindensis* Moffett 2007

182 ^f South African National Biodiversity Institute (SANBI) 2008

183

184 **Table 1** Plant and samango monkey dispersal traits, seed morphology and germination information reported in the literature for species used in our germination experiments

185 **2.1.3 Germination Experiments**

186 **2.1.3.1 Seed Treatments**

187 We applied five seed treatments that isolated specific biological processes, and a control (Control) of entire fruits
 188 for each seed species (Table 2). The control, in which seeds were left unchanged, allowed us to isolate the effect
 189 of the second treatment and fully evaluate the effect of seed handling mechanisms on germination (Samuels and
 190 Levey 2005; Baskin and Baskin 2014). The second treatment replicated the disinhibition effect (Disinhibition) of
 191 pulp removal either in the mouth or following gut passage, and allowed us to isolate the effects of seed-spitting
 192 and the chemical and/or mechanical effects of gut passage in proceeding treatments (Traveset and Verdú 2002;
 193 Samuels and Levey 2005; Fuzessy et al. 2016). The third treatment isolated mechanical scarification
 194 (Scarification) during oral processing and passage through gut from the first treatment and additional chemical
 195 scarification in the third treatment (Baskin and Baskin 2014). The fourth treatment was the gut passage effect
 196 (Gut-Passage), which allowed us to isolate the chemical processes and transit time that alter the seed during gut
 197 passage from the disinhibition and mechanical scarification effects of the second and third treatments respectively
 198 (Traveset and Verdú 2002; Samuels and Levey 2005). The fifth treatment was the fertiliser effect (Fertiliser)
 199 which allowed us to isolate the effect of the faecal matrix and measure the gut passage effect separately (Traveset
 200 and Verdú 2002). We only used the fourth and fifth treatments with *Ficus* spp. *S. chirindensis* seeds, as *S.*
 201 *cordatum* were not swallowed by samango monkeys. Lastly, the sixth treatment measured the added effect of
 202 desiccation following disinhibition on *S. cordatum* seeds (Desiccation), which we used to simulate dispersal into
 203 unfavourable dry habitats, with a potentially negative effect on germination, as these seeds are recalcitrant
 204 (Roberts 1973; Royal Botanic Gardens Kew 2017). Hereafter, we refer to treatments as Control, Disinhibition,
 205 Scarification, Gut-Passage, Fertiliser and Desiccation.

206

207

Treatment / Effect	Description / Seed Source	Species	Trials / Seeds in Each Nursery		
			Number of Trials	Length (days)	Seeds / Trial ^a
Control	Entire fruit, no treatment / fresh ripe fruit collected from different trees for each trial	<i>Ficus</i> spp.	5	105	40 fruits
		<i>Searsia chirindensis</i>	5	62	20 fruits
		<i>Syzygium cordatum</i>	4	102	20 fruits
Disinhibition	Manual removal of seed from pulp and sown within 24 h / fresh ripe fruit collected from different trees for each trial	<i>Ficus</i> spp.	5	105	40
		<i>Searsia chirindensis</i>	5	62	20
		<i>Syzygium cordatum</i>	4	102	20

Scarification	Manual removal of pulp and seed coat damaged by rubbing once along 100 mm length of medium-coarse sandpaper / fresh ripe fruit collected from different trees for each trial	<i>Ficus</i> spp.	5	105	40
		<i>Searsia chirindensis</i>	5	62	20
		<i>Syzygium cordatum</i>	4	102	20
Gut-Passage	Seeds collected from fresh faeces after passage through the monkeys' gut and washed in untreated water	<i>Ficus</i> spp.	5	105	40
		<i>Searsia chirindensis</i>	5	62	20
Fertiliser	Seeds left in freshly collected faecal matrix after passage through the monkeys' gut, and sown into the soil	<i>Ficus</i> spp.	5	105	20
		<i>Searsia chirindensis</i>	4	62	16 - 20
Desiccation	Seeds collected after being spat out by the monkeys and stored for 14 days	<i>Syzygium cordatum</i>	4	102	20

208 ^a *Ficus* spp. seeds were sown five per cell to control for hollow seeds used by fig wasps and we counted each cell as one seed.

209 **Table 2** Seed processing treatments of selected samango monkey-dispersed fruit species used in our germination
 210 experiments, including the number of seeds/fruits sown and number and duration of trials.

211

212 2.1.3.2 Seed Collection

213 We collected seeds used for Gut-Passage and Fertiliser treatments from fresh faecal samples and seeds for
 214 Desiccation treatment as the monkeys spat them out. To do this, we followed the samango monkey groups two
 215 days each week per group from dawn at their sleep site, until dusk when they settled at their sleep site. We
 216 collected fresh faecal samples from all age and sex classes *ad libitum* in separate plastic bags, noting the date and
 217 time of deposition on the bag. Faecal samples were stored overnight in dark sealed plastic boxes to keep them
 218 cool and processed early the following day. For the Fertiliser treatment, we visually inspected faecal samples for
 219 *Ficus* spp. or *S. chirindensis* seeds, removed all other seeds and divided the faecal sample into portions, ensuring
 220 identical numbers of sub-samples were used in each nursery. For *S. chirindensis*, we ensured seeds from faecal
 221 samples were sown in sufficient faecal matter to bury the seed into. Faecal samples not used in the Fertiliser
 222 treatment were gently washed, removing digesta and air-dried in a tent for 48 h. Once dry, we collected all entire
 223 and undamaged seeds from each sample, identified and counted them. We could only identify *Ficus* spp. to genus
 224 level, due to the high similarity and very small (< 2 mm) seed size. As *Ficus* spp. and *S. chirindensis* seeds are
 225 orthodox, we were able to store these seeds in paper bags in a sealed dark plastic box until needed, which we did
 226 for a maximum of ten days prior to sowing for the Gut-Passage treatment (Baskin and Baskin 2014).

227

228 We collected monkey-dispersed *S. cordatum* seeds immediately after being spat out, storing them for fourteen
 229 days also in paper bags in a sealed dark plastic box prior to sowing for the Desiccation treatment. These seeds are
 230 recalcitrant and we stored them prior to sowing to simulate dispersal into dry habitats, which the monkeys did in

231 their cheek pouches. We collected entire fresh mature fruits from monkey feeding trees, which we used for
232 Control, Disinhibition and Scarification treatments, processing them no longer than 24 h after collection. We
233 removed seeds from fruit pulp by hand and left seeds to air-dry at room temperature for 24 – 48 h (Baskin and
234 Baskin 2014), although seeds removed from *S. cordatum* fruit pulp were air-dried for a maximum of 24 h to avoid
235 desiccation. We scarified *S. chirindensis* and *S. cordatum* seeds by rubbing them once along a 15 x 15 cm piece
236 of medium-coarse sandpaper (Kimura and Islam 2012). Due to their small size, we scarified *Ficus* spp. seeds by
237 placing all seeds used per trial into a small plastic tub with a piece of the same sandpaper glued to each end and
238 shaking the tub in a circular motion for 5 min.

239

240 **2.1.3.3 Germination Trials**

241 We constructed two germination nurseries in an open area where there was no shading effect from trees or other
242 structures. We built a wooden frame and covered one with 80% shade cloth (shaded) and the other with 20%
243 shade cloth (unshaded) to simulate the difference in light conditions (hereafter Shading) within and outside of
244 continuous canopy cover into which the monkeys may disperse seeds respectively. Within each nursery, we
245 recorded temperature (°C) and relative humidity (%) at 30 min intervals using Easy-Log USB 2+ data loggers
246 (Lascar Electronics, Salisbury, UK) placed in the centre 1 m above ground level. We collected topsoil from a
247 transitional zone between the evergreen forest and semi-deciduous woodland. We collected sufficient topsoil for
248 all trials at once, in order to homogenise the substrate between trials and eliminate inconsistencies in soil texture,
249 porosity and chemical composition between trials. Soil pH was analysed by the Agricultural Research Council,
250 Institute for Soil, Climate and Water, Pretoria, South Africa, and was 6.76 and 6.52 in the shaded and unshaded
251 nursery respectively. We sieved the soil using a 1 mm mesh to remove large particles, seeds and plant matter,
252 mixed it with quarry sand (5-parts soil to 1-part sand), and stored it in a covered black plastic container until
253 required.

254

255 We sowed seeds in polystyrene germination trays (Plasgrow, Mpumalanga, South Africa), each containing 200
256 30 x 30 mm cells with a depth of 50 mm. We sowed one seed, faecal sample portion (Fertiliser) or entire fruit
257 (Control) per cell (Table 3) at a depth of 15 mm, except *Ficus* spp., for which we sowed five seeds per cell to
258 control for those used by fig wasps (Serio-Silva and Rico-Gray 2002), counting each cell as one seed. We sowed
259 entire *Ficus* spp. fruits at depths according to their individual size (Table 1), allowing for a covering of 15 mm of
260 soil. For all species, we marked the placement of the seed with a toothpick and undertook daily removal of

261 seedlings that were not at that location. We left seedlings that emerged next to the toothpick to grow until we
262 could identify them as our focal seed.

263

264 We used a nested experimental design whereby trays and cells in each nursery mirrored each other in terms of
265 species, treatments, faecal samples and positioning within the nursery, placing the germination trays on wooden
266 pallets 50 cm above the ground. We used one tray per trial in each nursery, with all seeds used for simulated
267 treatments originating from the same source tree in each trial. Primate dispersed *Ficus* spp. and *S. chirindensis*
268 seeds came from different faecal samples for each trial, although it was not always possible to use a single faecal
269 sample per trial, as they did not always contain sufficient quantities of seeds. We evenly watered trays in both
270 nurseries daily using a hosepipe and water piped directly from a stream in the forest, until the soil was wet to the
271 touch. Soil within the shaded nursery remained permanently moist for the duration of the germination trials, whilst
272 within the unshaded nursery, soil dried out daily between watering. We monitored germination daily and
273 considered the seed to have germinated upon first emergence of the cotyledon from the soil. Each trial was
274 terminated after the maximum published germination time (Table 1) plus either 10% or 14 days with no new
275 germination (Table 2). We did not determine the viability of ungerminated seeds after each trial.

276

277 **2.2 Data Analysis**

278 We conducted separate analyses for each species using Generalised Linear Mixed-Effects Models (GLMMs) in
279 R version 3.5.1. (R Core Team 2018) using the lme4 package (Bates et al. 2015). Recent reviews recommend
280 GLMMs as the preferred mode of analysis for germination data (Sileshi 2012), as the model allows analysis of
281 nested designs such as our dichotomous shade effect, as well as allowing for non-normal distributions of the
282 response variable and random effect specification (Schupp 1993; Bolker et al. 2009). In all cases, we modelled
283 seed treatment (four or five levels depending on species) and the interaction between treatment and shading (two
284 levels, shaded and unshaded) as fixed effects, to evaluate separately the effects of each treatment on the probability
285 of germination (binomial) and germination latency (number of days taken to germinate). We included trial nested
286 in shading (or nursery) as a random effect to control for spatiotemporal variation across trials, as we could not
287 sow all trials at the same time due to accessible fruit availability across the study site. We verified parsimony of
288 the model against a reduced model omitting the interaction term, followed by a null model of the random effect
289 only using a likelihood ratio test. We assessed optimal model adequacy by examining standard residuals vs. fitted
290 residuals and graphical distribution of errors and conducted Wald Chi-Square tests to determine the significance

291 of the fixed effects at $p = 0.05$. To isolate the effects of the different treatments on seed germination, we conducted
292 Least-Squares Means analysis of the final model using the emmeans package (Lenth 2018) and performed
293 pairwise Tukey' HSD comparisons using the multcomp package (Hothorn et al. 2008), to compare treatments
294 with control seeds.

295

296 **3. RESULTS**

297 **3.1 Probability of Germination**

298 We had a total germination success of 57.1% in *Ficus* spp., 25.3% in *S. chirindensis* and 53.9% in *S. cordatum*
299 seeds. There was no interaction effect between shading and treatment on the probability of germination in all three
300 fruit-tree species (Table 3). However, there was a significant effect of seed processing treatment on the probability
301 of germination (Table 3) and significant pairwise differences between treatments (Fig. 1). All pairwise
302 comparisons with Control were significant except for Fertiliser in *Ficus* spp. and Gut-Passage and Fertiliser in *S.*
303 *chirindensis*. Non-significant pairwise comparisons are given in Supplementary Information Table B.

304

305 For both *Ficus* spp. and *S. chirindensis* seeds, our analyses indicated Disinhibition and Scarification treatments
306 had the highest probability of germination, both of which were significantly higher than Controls, but not different
307 from each other (Tukey's HSD test $Z = 1.091$, $p = 0.81$ and $Z = -3.03$, $p = 0.99$, respectively). In *Ficus* spp. seeds,
308 Gut-Passage significantly reduced the probability of germination from Controls, and Fertiliser significantly
309 increased the probability of gut passed seeds germinating, however our analyses indicated this was not
310 significantly different from Controls (Tukey's HSD test $Z = 2.12$, $p = 0.21$). For *S. chirindensis*, we found neither
311 Gut-Passage nor Fertiliser significantly affected the probability of seeds germinating compared with Controls
312 (Tukey's HSD test $Z = -0.95$, $p = 0.87$ and $Z = -1.42$, $p = 0.6$ respectively), and Fertiliser had no additional benefits
313 to Gut-Passage (Tukey's HSD test $Z = -0.56$, $p = 0.98$). In *S. cordatum*, our analyses indicated Disinhibition
314 significantly increased the probability of germination compared with Controls, although our analyses also
315 indicated a considerable reduction in the benefit of Disinhibition following Desiccation. Compared with
316 Disinhibition, Scarification reduced the probability of germination, although not significantly (Tukey's HSD test
317 $Z = 2.48$, $p = 0.05$).

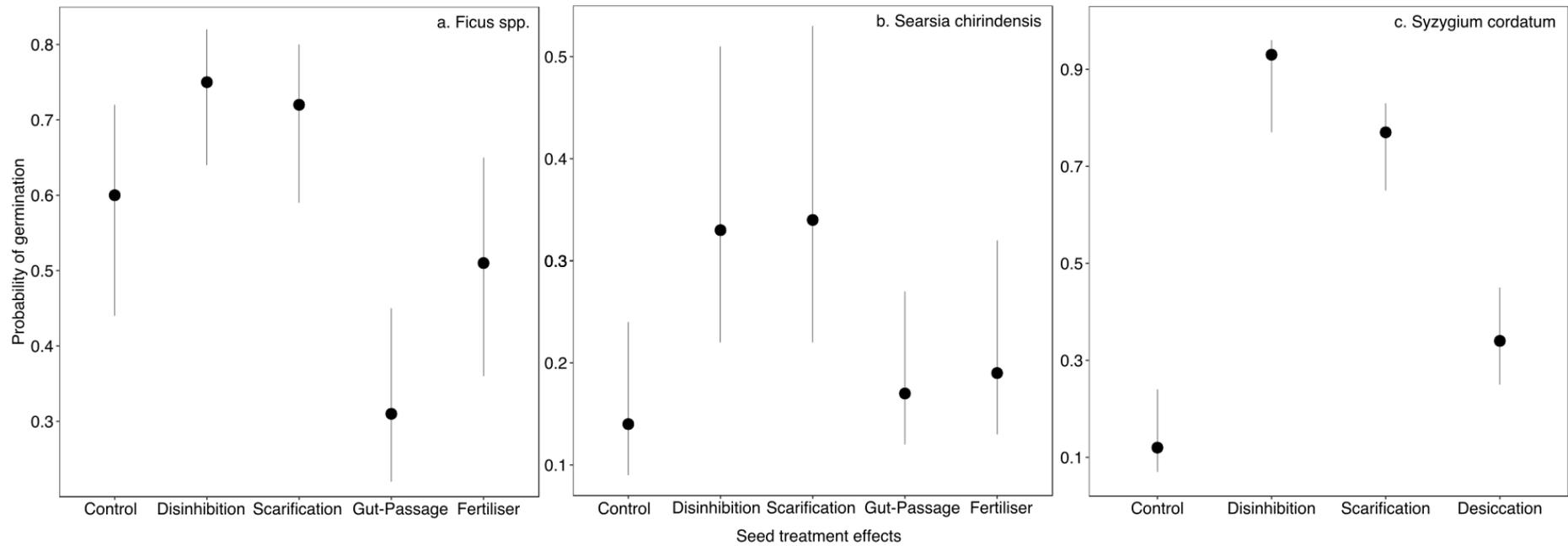
318

319

GLMM Wald Chi-square Test				
Species	Effect	χ^2	df	p-value
<i>Ficus</i> spp.	Shading : Treatment	10.02	5	0.07
	Treatment	112.14	4	<0.001
<i>Searsia chirindensis</i>	Shading : Treatment	2.65	5	0.75
	Treatment	35.49	4	<0.001
<i>Syzygium cordatum</i>	Shading : Treatment	2.67	4	0.16
	Treatment	61.88	3	<0.001
Significant Tukey's HSD Pairwise Comparisons				
	Effect Comparison	Estimate \pm SE	Z-value	p-value
<i>Ficus</i> spp.	Control – Disinhibition	-0.7 \pm 0.17	-4.2	<0.001
	Control – Scarification	-0.52 \pm 0.16	-3.27	0.009
	Control – Gut-Passage	0.99 \pm 0.15	6.5	<0.001
	Disinhibition – Gut-Passage	1.7 \pm 0.16	9.45	<0.001
	Disinhibition – Fertiliser	0.99 \pm 0.16	6.32	<0.001
	Scarification – Gut-Passage	1.52 \pm 0.17	8.77	<0.001
	Scarification – Fertiliser	0.81 \pm 0.15	5.45	<0.001
	Gut-Passage – Fertiliser	-0.71 \pm 0.14	-4.98	<0.001
<i>Searsia chirindensis</i>	Control – Disinhibition	-1.6 \pm 0.41	-3.86	<0.001
	Control – Scarification	-1.66 \pm 0.42	-3.98	<0.001
	Disinhibition – Gut-Passage	1.14 \pm 0.31	3.65	0.002
	Disinhibition – Fertiliser	0.92 \pm 0.31	2.96	0.02
	Scarification – Gut-Passage	1.2 \pm 0.32	3.8	0.001
	Scarification – Fertiliser	0.99 \pm 0.32	3.12	0.01
<i>Syzygium cordatum</i>	Control – Disinhibition	-7.2 \pm 1.52	-4.75	<0.001
	Control – Scarification	-3.75 \pm 0.68	-5.54	<0.001
	Control – Desiccation	-2.06 \pm 0.65	-3.17	0.007
	Disinhibition – Desiccation	5.25 \pm 1.38	3.72	<0.001
	Scarification – Desiccation	1.69 \pm 0.28	5.94	<0.001

320

321 **Table 3** Model output of the GLMM Wald Chi-square test on the effect of treatment and the interactive effect of
322 shading (shaded/unshaded) and treatment, with Tukey's HSD pairwise comparison tests on the probability of
323 germination for selected samango monkey-dispersed fruit species' seeds. Treatments: Control, entire fruit, no
324 treatment; Disinhibition, effect of pulp removal; Scarification, disinhibition *plus* mechanical scarification of seed
325 coat; Gut-Passage, disinhibition *plus* mechanical *and* chemical scarification during gut passage (*Ficus* spp. and *S.*
326 *chirindensis*); Fertiliser, gut passage effect *plus* fertiliser effect of the faecal matrix (*Ficus* spp. and *S.*
327 *chirindensis*); Desiccation, disinhibition *plus* desiccation (*S. cordatum*).



328

329 **Fig. 1** Least squares means probability of germination (\pm 95% CI) of selected samango monkey-dispersed fruit species' seeds subject to different processing treatments.

330 Treatments: Control, entire fruit no treatment; Disinhibition, effect of pulp removal; Scarification, disinhibition *plus* mechanical scarification of seed coat; Gut-Passage,

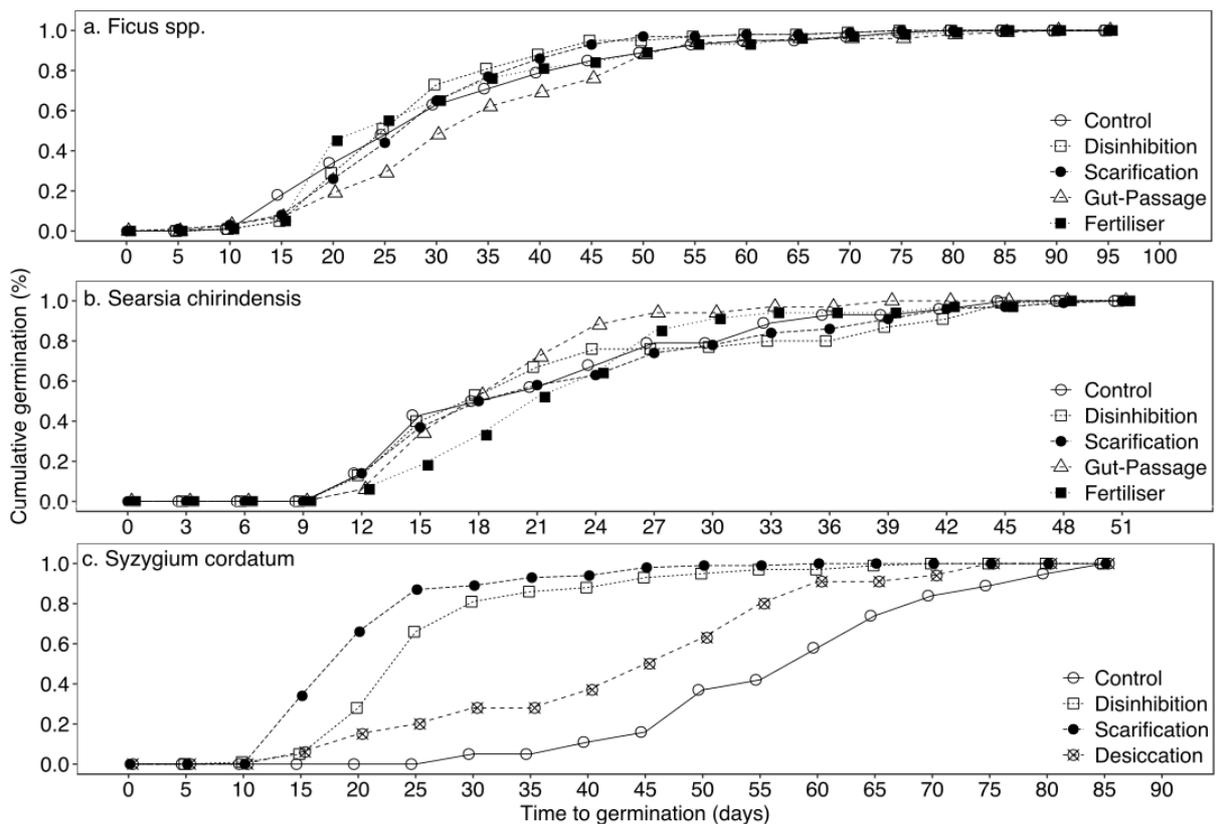
331 disinhibition *plus* mechanical *and* chemical scarification; Fertiliser, gut passage effect *plus* fertiliser effect of the faecal matrix; Desiccation, disinhibition *plus* desiccation.

332

333 **3.3 Germination Latency**

334 We observed similar germination patterns between all treatments in *Ficus* spp. and *S. chirindensis* seeds, with
 335 50% germination occurring between 25 – 30 and 18 – 20 days respectively (Fig. 2). We observed similar patterns
 336 in germination between two pairs of treatments in *S. cordatum*, with 50% germination occurring after 21 and 16
 337 days in Disinhibition and Scarification treatments respectively, and after 57 and 45 days in Controls and
 338 Desiccation treatments respectively (Fig. 2).

339



340 **Fig. 2** Cumulative germination (%) of selected samango monkey-dispersed fruit species' seeds subject to different
 341 processing treatments. Treatments: Control, entire fruit no treatment; Disinhibition, effect of pulp removal;
 342 Scarification, disinhibition *plus* mechanical scarification of seed coat; Gut-Passage, disinhibition *plus* mechanical
 343 *and* chemical scarification; Fertiliser, gut passage effect *plus* fertiliser effect of the faecal matrix; Desiccation,
 344 disinhibition *plus* desiccation
 345

346
 347 Our analyses indicated significant interaction effects between treatment and shading on germination latency for
 348 *Ficus* spp. and *S. cordatum* seeds (Table 4); however, we found no significant effect of shading on germination
 349 latency within treatments in either of these species (Fig. 3). There was no significant interaction effect between

350 shading and treatment in *S. chirindensis*. Non-significant pairwise comparisons are given in Supplementary
 351 Information Table B.

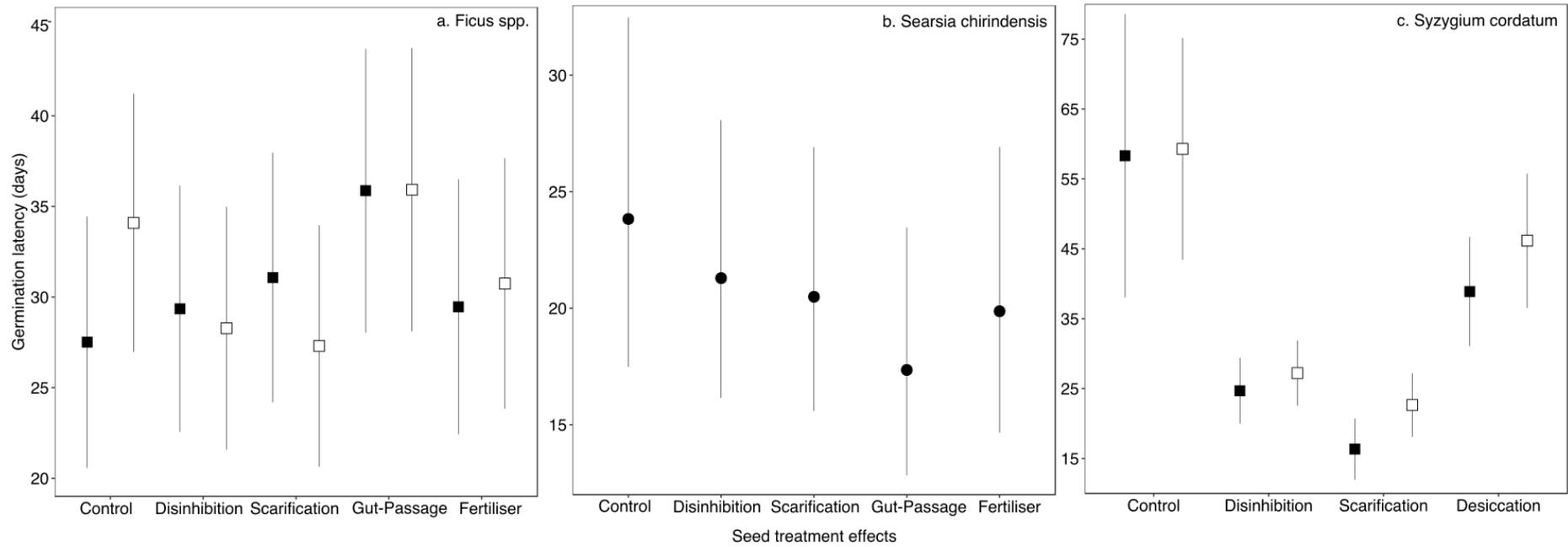
GLMM Wald Chi-square Test							
Species	Effect	χ^2	df	<i>p</i> -value			
<i>Ficus</i> spp.	Shading : Treatment	2192.6	5	<0.001			
	Treatment	3376.8	5	<0.001			
<i>Searsia chirindensis</i>	Shading : Treatment	9.22	5	0.1			
	Treatment	12.38	4	0.01			
<i>Syzygium cordatum</i>	Shading : Treatment	12.3	4	0.02			
	Treatment	142.52	3	<0.001			
Significant Tukey's HSD Pairwise Comparisons							
	Shading	Effect Comparison	Estimate \pm SE	Z-value	<i>p</i> -value		
<i>Ficus</i> spp.	Shaded	Control – Gut-Passage	-8.35 \pm 1.97	-4.23	<0.001		
		Disinhibition – Gut-Passage	-6.51 \pm 1.91	-3.42	0.02		
		Gut-Passage – Fertiliser	6.4 \pm 2.05	3.12	0.04		
	Unshaded	Control – Disinhibition	5.81 \pm 1.61	3.62	0.008		
		Control – Scarification	6.79 \pm 1.61	4.23	<0.001		
		Disinhibition – Gut-Passage	-7.61 \pm 1.96	-3.9	0.003		
		Scarification – Gut-Passage	-8.62 \pm 1.96	-4.4	<0.001		
	Unshaded : Shaded ^a		Not Significant				
<i>Searsia chirindensis</i>	Shading not significant						
		Control – Gut-Passage	0.32 \pm 0.09	3.35	0.007		
<i>Syzygium cordatum</i>	Shaded	Control – Disinhibition	32.63 \pm 7.37	4.57	<0.001		
		Control – Scarification	41.97 \pm 7.35	7.71	<0.001		
		Disinhibition – Scarification	8.35 \pm 1.23	6.81	<0.001		
		Disinhibition – Desiccation	-14.2 \pm 7.35	2.68	<0.001		
		Scarification – Desiccation	-22.55 \pm 7.35	2.6	<0.001		
	Unshaded	Control – Disinhibition	32.07 \pm 5.75	5.57	<0.001		
		Control – Scarification	36.63 \pm 5.74	6.38	<0.001		
		Disinhibition – Scarification	4.56 \pm 1.45	3.12	0.03		
		Disinhibition – Desiccation	-18.95 \pm 3.43	-5.53	<0.001		
		Scarification – Desiccation	-23.5 \pm 3.43	-6.86	<0.001		
		Unshaded : Shaded ^a		Control – Disinhibition	34.6 \pm 6.06	5.71	<0.001
				Control – Scarification	42.95 \pm 6.03	7.12	<0.001
				Control – Desiccation	20.4 \pm 6.48	3.15	0.03
			Disinhibition – Control	-31.1 \pm 7.63	-4.08	<0.001	
			Disinhibition – Scarification	10.87 \pm 2.35	4.64	<0.001	
			Disinhibition – Desiccation	-11.67 \pm 3.34	-3.5	0.008	
			Scarification – Control	-35.65 \pm 7.61	-4.68	<0.001	
			Scarification – Desiccation	-16.23 \pm 3.31	-4.9	<0.001	
		Desiccation – Disinhibition	21.48 \pm 3.92	5.48	<0.001		
		Desiccation – Scarification	29.82 \pm 3.87	7.71	<0.001		

352 ^aThe first treatment listed was in unshaded conditions

353 **Table 4** Model output of the GLMM Wald Chi-square test on the effect of treatment and the interactive effect of
 354 shading (shaded/unshaded) and treatment, with significant Tukey's HSD pairwise comparison tests on
 355 germination latency for selected samango monkey-dispersed fruit species' seeds. Treatments: Control, entire fruit,
 356 no treatment; Disinhibition, effect of pulp removal; Scarification, disinhibition *plus* mechanical scarification of
 357 seed coat; Gut-Passage, disinhibition *plus* mechanical *and* chemical scarification during gut passage (*Ficus* spp.

358 and *S. chirindensis*); Fertiliser, gut passage effect *plus* fertiliser effect of the faecal matrix (*Ficus* spp. and *S.*
359 *chirindensis*); Desiccation, disinhibition *plus* desiccation (*S. cordatum*).

360



361

362 **Fig. 3** Least squares means germination latency (\pm 95% CI) of selected samango monkey-dispersed fruit species' seeds subject to different processing treatments. Treatments:
363 Control, entire fruit no treatment; Disinhibition, effect of pulp removal; Scarification, disinhibition *plus* mechanical scarification of seed coat; Gut-Passage, disinhibition *plus*
364 mechanical *and* chemical scarification; Fertiliser, gut passage effect *plus* fertiliser effect of the faecal matrix; Desiccation, disinhibition *plus* desiccation. Interactive effect of
365 treatment & shading: ● no significant interacting effect; ■ significant interactive effect shaded, □ significant interactive effect unshaded).

366

367 In shade, our analyses indicated that in *Ficus* spp., Gut-Passage significantly increased germination latency
 368 compared with Disinhibition and Fertiliser treatments and Controls. Unshaded, our analyses indicated that both
 369 Disinhibition and Scarification significantly reduced germination latency compared with the other treatments and
 370 Controls. We found no significant interacting effects between treatment and shading for *S. chirindensis* seeds, and
 371 our analyses indicated a significant difference in germination latency between Controls and Gut-Passage, the latter
 372 of which germinated fastest (Table 4). All three treatments reduced germination latency of *S. cordatum* seeds
 373 compared with Controls, which we found took the longest to germinate. Our analyses indicated that Scarification
 374 significantly reduced germination latency compared with all other treatments, with those sown in shade
 375 germinating fastest.

376

377 3.4 Exclusion of Ecologically Relevant Seed Treatments

378 To identify if exclusion of ecologically relevant seed treatments or relevant controls would affect our
 379 interpretations of the impact of the disperser on seed germination, we considered the positive, negative or neutral
 380 effects our disperser had on the probability of seed germination (Table 5). Firstly, we considered exclusion of
 381 entire fruit control groups (Controls), instead assessing the difference in probability of germination between
 382 manual removal of seeds from mature fruits (Disinhibition) and seeds either removed from faecal samples (Gut-
 383 Passage, *Ficus* spp. and *S. chirindensis*), or collected after being spat out (Desiccation, *S. cordatum*). Our analysis
 384 indicated negative disperser effects in each of these comparisons as compared with Disinhibition the probability
 385 of germination in seeds from Gut-Passage and Desiccation treatments was significantly lower. When we included
 386 Controls, disperser effects were positive (Disinhibition and Desiccation) for *S. cordatum*, positive (Disinhibition)
 387 and neutral (Gut-Passage) for *S. chirindensis*, and positive (Disinhibition) and negative (Gut-Passage) for *Ficus*
 388 spp. seeds. Secondly, we considered the disperser effect when we include the effect of the faecal matrix (Fertiliser)
 389 in species with gut-passed seeds. Our analysis indicated neutral effects when compared with both Controls and
 390 Gut-Passage in *S. chirindensis*, and neutral and positive effects when compared with Controls and Gut-Passage
 391 respectively in *Ficus* spp. seeds.

392

Species	Comparison	GLMM Tukey's HSD Pairwise Comparisons			Impact on Germination
		Z-Value	p-Value	Difference	
Exclusion of Entire Fruit Control and Fertiliser Effect					
<i>Ficus</i> spp.	Disinhibition – Gut-Passage	9.45	<0.001	- 44.28 %	Negative
<i>Searsia chirindensis</i>	Disinhibition – Gut-Passage	3.65	0.002	- 16.2 %	Negative
<i>Syzygium cordatum</i>	Disinhibition – Desiccation	3.72	<0.001	- 59.46 %	Negative
Inclusion of Entire Fruit Control					

<i>Ficus</i> spp.	Control – Disinhibition	-4.2	<0.001	+ 15.57 %	Positive
	Control – Gut-Passage	6.5	<0.001	- 28.71 %	Negative
<i>Searsia chirindensis</i>	Control – Disinhibition	-3.86	<0.001	+ 19.22 %	Positive
	Control – Gut-Passage	-0.95	0.87	+ 3.02 %	Neutral ^a
<i>Syzygium cordatum</i>	Control – Disinhibition	-4.75	<0.001	+ 81.59 %	Positive
	Control – Desiccation	-3.17	0.007	+ 22.1 %	Positive
Inclusion of Fertiliser Effect					
<i>Ficus</i> spp.	Control – Fertiliser	2.12	0.21	- 8.84 %	Neutral ^a
	Gut-Passage – Fertiliser	-4.98	<0.001	+ 19.87 %	Positive
<i>Searsia chirindensis</i>	Control – Fertiliser	-1.42	0.6	+ 4.9 %	Neutral ^a
	Gut-Passage – Fertiliser	-0.56	0.98	+ 1.88 %	Neutral ^a

393 ^a Non-significant difference

394 **Table 5** Comparison between exclusion/inclusion of entire fruit control groups and/or the fertiliser effect, using
395 model output of the GLMM Tukey’s HSD pairwise comparison tests on the probability of germination for selected
396 samango monkey-dispersed fruit species’ seeds. Treatments: Control, entire fruit, no treatment; Disinhibition,
397 effect of pulp removal; Gut-Passage, disinhibition *plus* mechanical *and* chemical scarification during gut passage
398 (*Ficus* spp. and *S. chirindensis*); Fertiliser, gut passage effect *plus* fertiliser effect of the faecal matrix (*Ficus* spp.
399 and *S. chirindensis*); Desiccation, disinhibition *plus* desiccation (*S. cordatum*).

400

401 **4. DISCUSSION**

402 In a review conducted in 2005, 77% of germination studies omitted using entire fruits as controls in experiments
403 assessing the effect of gut passage on seed germination (Samuels and Levey 2005). In our review of mammalian
404 germination studies, 76% of studies used manually extracted seeds as controls demonstrating a lack of
405 progression. Furthermore, 76% of studies excluded the fertiliser effect and only 9% included mechanical
406 scarification of de-pulped seeds along-side gut-passed seeds. Without isolating each fundamentally different
407 mechanism, the absolute effect of frugivores on seed germination is confounded (Samuels and Levey 2005;
408 Robertson et al. 2006; Baskin and Baskin 2014) and these studies are weakened in their conclusions. Our analyses
409 indicated that samango monkeys are effective in the qualitative side of seed dispersal, as seed processing
410 mechanisms had a positive and/or neutral influence on germination potential of seeds compared with entire fruits
411 where seeds were untreated. As is widely reported (Robertson et al. 2006), removal of seeds from fruit pulp (*the*
412 *disinhibition effect*) had the greatest positive effect on the probability of germination in each of the selected fruit
413 species we assessed. Excluding entire fruit as control groups from our analyses altered the conclusions drawn
414 from our data, thus underestimating the influence seed handling by samango monkeys has on seed germination.
415 As such, our results concur with others, and reiterate the need for inclusion of all ecologically relevant treatments

416 that simulate dispersal modes of frugivores, to estimate accurately their role in seed dispersal (Samuels and Levey
417 2005; Baskin and Baskin 2014; Fuzessy et al. 2016).

418

419 **4.1 Effect of samango monkeys on seed germination**

420 The disinhibition effect had a significant positive influence on the probability of germination compared with
421 control groups of entire fruits in each of the fruit species we assessed, suggesting that as seed-spitters samango
422 monkeys have an important role in their dispersal. This was most noticeable in *S. cordatum* where we observed
423 samango monkeys to disperse seeds via spitting as they were consuming fruits in the parent tree or following
424 storage in cheek pouches. Our results are similar to previous research conducted on *Syzygium legatii* (mountain
425 water-berry) seeds spat out by samango monkeys (B. Linden, unpublished data), as well as studies on *Syzygium*
426 spp. in other taxa (e.g. Albert et al., 2013; Gross-Camp and Kaplin, 2005; Sengupta et al., 2014). The role of
427 samango monkeys in *S. cordatum* seed dispersal may also be greatly dependent on where they deposit seeds, as
428 shown by the significant reduction in the probability of germination of seeds we subjected to desiccation,
429 compared to freshly de-pulped seeds. As recalcitrant seeds are sensitive to desiccation (Roberts 1973), it may be
430 that *S. cordatum* relies on seed-spitters to remove pulp and that germination must occur soon after before seeds
431 desiccate. Mature *S. cordatum* trees are typically located close to water and dispersal under or close to parent
432 trees, where the environment can slow the desiccation process, may confer an advantage to seeds. Compared with
433 the disinhibition effect, we found scarification to have a non-significant negative effect on the probability of
434 germination and a significant reduction on germination latency in *S. cordatum*. Damage to the protective seed
435 coat through scarification or removal has been shown to reduce cumulative germination in *S. cordatum* (Prins and
436 Maghembe 1994). Guenon cheek pouches contain a high level of α -amylase, a saliva enzyme involved in pre-
437 digestion breakdown of starch (Murray 1975), which may reduce the need for high-molar mastication and
438 therefore scarification damage to seeds in soft-pulped fruits. The significant benefits in increased germination and
439 reduced latency to *S. cordatum* seeds through pulp removal are likely to help these seeds germinate and establish
440 during the wet season. This is further evident in the significantly reduced benefits we observed when we dried
441 spat seeds prior to sowing

442

443 While the disinhibition effect had positive effects on germination in each of the species we assessed, the gut
444 passage effect was negative in *Ficus* spp. and neutral in *S. chirindensis*. In *Ficus* spp., the gut passage effect
445 significantly reduced the probability of germination, and increased germination latency, more than every other

446 treatment. The effect of gut passage on *Ficus* spp. seeds reported in the literature are highly variable, with positive
447 (e.g. Chen et al., 2017; Figueiredo, 1993; Mosallam, 1996; Oleksy et al., 2017), negative (e.g. Chang et al., 2016;
448 Compton et al., 1996; Tsuji et al., 2017) and neutral (e.g. Heer et al., 2010; Maccarini et al., 2018; Stevenson et
449 al. 2002) effects reported from a wide range of frugivorous mammals. Previous research on samango monkeys
450 found the gut passage effect significantly reduced germination of *Ficus* spp. seeds compared to the disinhibition
451 effect but had no significant effect on *Keetia gueinzii* (climbing turkey-berry) seeds (B. Linden, unpublished data).
452 In *S. chirindensis*, we found the gut passage effect on seed germination was neutral compared with entire fruit
453 controls, although the gut passage effect significantly reduced germination latency. Again, results reported in the
454 literature for *Searsia* spp. are variable (e.g. Foord et al., 1994; Mosallam, 1996).

455

456 The highly variable results reported for these species suggest there are likely to be species- and/or site-specific
457 variables not typically measured in mammalian frugivore germination experiments that influence seed
458 germination following gut passage. These may include chemicals in the gastrointestinal (GI) tract that may vary
459 in respect to dietary intake of dispersers, as well as anatomical differences of the GI tract that may increase the
460 passage time of seeds and therefore time available for chemical and mechanical scarification (Traveset et al.
461 2007). However, while germination latency increased in *Ficus* spp., seeds were still viable following passage
462 through the gut, and in *S. chirindensis*, gut-passage significantly reduced germination latency.

463

464 More surprisingly, in *Ficus* spp., the fertiliser effect increased germination success and reduced germination
465 latency compared to gut passage alone, and this effect was neutral compared to entire fruit controls. We observed
466 a similar neutral effect in *S. chirindensis*. The majority of mammalian frugivore germination studies remove seeds
467 from faecal samples to test the gut passage effect and the literature including the fertiliser effect is scarce. In our
468 literature search, 93% of mammalian frugivore germination studies omitted the fertiliser effect. Where the effect
469 is included, results are again variable (Heer et al., 2010; Rojas-Martinez et al., 2015; Tutin et al., 1991; Valenta
470 and Fedigan, 2009). Lethal effects reported in some studies (e.g. Anzures-Dadda et al., 2016) may result from
471 seeds being removed from faecal samples and washed prior to re-covering with fresh faeces, increasing
472 susceptibility to fungal attack (Wang and Smith 2002).

473

474 Our *in-situ* germination experiments were subject to natural variation of abiotic factors: temperature, humidity
475 and sunlight levels and are in contrast to *ex-situ* studies conducted under controlled conditions (e.g. Anto et al.,

476 2018; Figueiredo, 1993; Kankam and Oduro, 2012). Significant differences between the gut passage and
477 disinhibition effects in field experiments are rarely reported, with the majority of positive influences coming from
478 studies conducted in laboratory settings (Fuzessy et al. 2016). The significant decrease in the probability of
479 germination we report from gut passed *Ficus* spp. seeds requires further investigation.

480

481 *Ficus* spp. fruits are considered keystone resources for many animal species including primates (Estrada and
482 Coates-Estrada 1984), as their typical asynchronous year-round fruiting can be a reliable food source (Bleher et
483 al. 2003). Figs are synconium (Galil 1977) containing hundreds of small (<2 mm) imbedded seeds (Lambert
484 1999). The lengthy processing time it would take to orally remove the seeds means samango monkeys are likely
485 to swallow more than they spit out (Lambert 1999). As such, *Ficus* spp. may trade reduced quality of dispersal of
486 gut-passed seeds for quantity, relying instead on the unpredictable heterogeneity of the environment into which
487 samango monkeys disperse seeds (Chang et al. 2016). Similarly, *S. chirindensis* may rely on the reduced
488 germination latency of gut-passed seeds to help them establish. Like other Cercopithecines, samango monkeys
489 consume unripe fruit (Lawes 1991; Linden et al. 2015) acting as seed predators (Wrangham et al. 1998). As such,
490 low germination of seeds we collected from samango monkey faeces may be because they came from unripe fruit.
491 Alternatively, the chemical and/or mechanical scarification in the gut may damage the embryo (Samuels and
492 Levey 2005). Embryos inside the *Ficus* spp. seeds may also have been destroyed by fig wasps (Figueiredo 1993;
493 Righini et al. 2004).

494

495 **4.2 Exclusion of ecologically relevant seed treatments**

496 The majority of mammalian frugivore germination studies generated from our literature search exclude
497 ecologically relevant treatments from their study design. More than 75% of studies did not use entire fruits as
498 control groups in germination studies, instead comparing germination of gut passed seeds removed from faeces
499 with seeds manually removed from fruit pulp. This approach has several issues. Firstly, as discussed by Samuels
500 and Levey (2005), removal of fruit pulp is already a mechanism that can induce germination by removing
501 chemicals within fruit pulp that act as germination inhibitors (the *disinhibition effect*). Therefore the effect of gut
502 passage being assessed is the mechanical and/or chemical alteration (scarification) of the seed coat and not the
503 combined effect of disinhibition *plus* gut passage, which is important as each of these mechanisms can influence
504 germination independently (e.g. Baskin and Baskin, 2014). Secondly, in this approach there are no other treatment

505 against which the mechanical and chemical scarification of the seed coat can be assessed, further weakening
506 interpretation of the effect of frugivores on seed germination, extending to their role in seed dispersal.

507

508 In the present study, excluding entire fruit control groups, instead comparing germination response of seeds
509 subject with the disinhibition effect and gut passage (*Ficus* spp. and *S. chirindensis*) or desiccation (*S. cordatum*)
510 effect, influenced the interpretation of our analyses: we interpreted the disperser as having a negative impact on
511 seed dispersal, as the probability of germination was significantly lower for the gut passage and desiccation effects
512 than for the disinhibition effect. On the other hand, our analyses, which included entire fruit as the control group,
513 indicated that samango monkeys are effective in the qualitative side of seed dispersal, as seed processing
514 mechanisms had positive and/or neutral effects on the germination potential of seeds. Including entire fruits in the
515 analysis meant that we isolated scarification in the gut from disinhibition and were able to include an assessment
516 of the ecological role seed spitting plays on seed germination.

517

518 In addition to the gut passage effect, we included a separate treatment in our germination experiments to isolate
519 the chemical and mechanical effects of gut passage. The term ‘scarification effect’ is used ambiguously in the
520 current literature to describe the chemical and mechanical alteration of the seed coat during gut-passage (Samuels
521 and Levey 2005; Traveset et al. 2007). However, it does not distinguish chemical treatments, which can alter and
522 soften the seed coat, from mechanical action, which can physically damage it (Baskin and Baskin 2014). Instead,
523 each dispersal mechanism should be assessed against a control whereby the disperser has no influence on seed
524 fate, as well as identifying a positive or negative cumulative effect from the previous treatment (Traveset and
525 Verdú 2002; Samuels and Levey 2005; Robertson et al. 2006). Only 9% of mammalian frugivore germination
526 studies generated from our literature search included manual chemical and/or mechanical scarification of de-
527 pulped seeds alongside gut-passed seeds, thereby isolating scarification processes. In the present study, we found
528 the probability of manually scarified seeds germinating was significantly higher than gut passed *Ficus* spp. and *S.*
529 *chirindensis* seeds. It may be that the methods we employed to scarify seeds were insufficient to damage the seed
530 coat to the same extent as passage through the gut, or it may be that chemical scarification in samango monkey’s
531 gut reduces the germination potential of seeds.

532

533 We found that in *Ficus* spp., the faecal matrix significantly increased the probability of gut-passed seeds
534 germinating. We found a similar, albeit negligible, effect in *S. chirindensis*, and it may be that digestive acids

535 contained within the faecal matrix continue to alter the seed coat after defaecation. Alternatively, the embryo may
536 be able to extract nutrients from the faeces, which were no longer available once we had removed seeds from
537 faeces. Either way, inclusion of the fertiliser effect has significant consequences on our interpretation of the
538 influence seed handling by samango monkeys has in seed germination. Without it, especially in *Ficus* spp., the
539 role of samango monkeys would have been significantly underestimated.

540

541 **4.3 Implications for future studies**

542 The results we obtained in *Ficus* spp. require further investigation, not least as they are in contrast to results
543 reported in other studies. A potential limitation of our study was our analysis of the three *Ficus* spp. together
544 because we could not distinguish each species' seeds extracted from faecal samples. Pooling these data meant that
545 any interpretation of our findings is at the genus level and not species-specific. Whilst we acknowledge that this
546 may have introduced bias into the germination experiments of *Ficus* spp. seeds, we took care to assign seeds and
547 entire fruits randomly, to each treatment and each trial, to minimise such bias. We further suggest that germination
548 experiments investigating the effect of gut passage on *Ficus* spp. seeds should share seeds from faecal samples
549 between the gut passage and fertiliser effect treatments. The seeds we used for each of these treatments came from
550 different faecal samples and as a result, we did not isolate the fertiliser effect using seeds from the same faecal
551 sample and could not control for individual variation of digestive enzymes and gut-passage time. The dispersal
552 mode of seed swallowing may be a precursor for secondary dispersers, such as dung beetles (Culot et al. 2018),
553 and longitudinal studies incorporating secondary dispersal may elucidate to this. Our treatments for *S. cordatum*
554 isolated the disinhibition effect and the effect of desiccation; however, we did not isolate the potential effects of
555 individual variation in saliva chemicals on germination. We suggest that future studies should aim to use freshly
556 de-pulped and spat out seeds in both the disinhibition and desiccation treatments.

557

558 **4.4 Conclusion**

559 Samango monkeys spend most of their time in forests compared with other South African primates and they are
560 highly dependent on high-canopy evergreen forest (Linden et al. 2015). As both seed-spitters and seed-
561 swallowers, samango monkeys have multiple and important roles in seed dispersal by influencing the germination
562 probability and latency of seeds contained within the fruits they consume. Our study addressed these roles by
563 including in our germination experiments fruit species whose seeds they disperse through both roles. Our study
564 also demonstrated that to understand the role of frugivores in vegetation dynamics, experiments that assess the

565 influence of seed handling on seed germination should include ecologically relevant treatments that isolate each
566 handling mechanisms' cumulative effect, as well as a meaningful control. Germination is only one component of
567 successful establishment of a plant in a suitable microsite, the others being the method of transport and successful
568 arrival in that site, as well as continued delivery of niche space to maintain a plant throughout its life cycle.
569 However, our study demonstrates that the impacts of frugivores on germination may be under- or over-estimated
570 in ecological literature where ecologically relevant treatments and meaningful controls are excluded. There is,
571 therefore, a distinct gap in the literature aimed at understanding the substantial role frugivores have in maintaining
572 the resilience of ecosystems exposed to continued degradation.

573

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581

582 **Ethical Statement**

583 All procedures performed in studies involving human participants were in accordance with the ethical standards
584 of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later
585 amendments or comparable ethical standards. All applicable institutional and/or national guidelines for the care
586 and use of animals were followed.

587

588 **Declarations of interest:** none

589

590 **5. REFERENCES**

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