

Does the seed disperser matter? The influence of dispersal type on survival of *Araucaria angustifolia* seeds

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1 **Does the seed disperser matter? The influence of dispersal type on survival of *Araucaria angustifolia* seeds**

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31 Summary

32 Zoochoric dispersion is fundamental for the colonization of habitats by plants with large and heavy seeds such as the
33 Paraná pine (*Araucaria angustifolia*). This is an endangered conifer from South America whose recruitment is heavily
34 impacted by animals, but the way that different zoochoric dispersal modes and deposition sites can affect its successful
35 establishment is not known yet. Thus, in this study, we aimed to evaluate the effect of dispersal mode (accordingly to
36 the seed disperser), distance from adult conspecifics, and disposition site on long-term recruitment success. The
37 experimental design included two environments (forest and open field) and each of them received 30 micro-habitat
38 sampling stations (simulating deposition sites: 10 under conspecific; 10 far from conspecifics, next to a landmark; and
39 10 far from conspecifics, without a landmark), and each microhabitat had three levels of dispersal type simulating
40 animal treatment (intact buried seed; intact seed over the ground; partially preyed seed over the ground), each with
41 five seeds, totaling 900 seeds. In the forest environment, an experiment was also conducted to verify the fate of seeds
42 using the spool and line technique. The environment and the micro-habitat alone did not explain seed survival,
43 although the dispersal type given by the animal disperser was significant for survival and the buried seeds were the
44 only ones that survived until the last survey. With the spool and line seed experiment, we attested that most of the
45 Paraná pine seeds were preyed after removal (81.5%), and only buried seeds survived, reinforcing the role of scatter-
46 hoarding animals as important agents in the Paraná pine dispersal. This way, our results showed that Paraná pine seeds
47 suffer a very high predation rate, and that only a few seeds escape from predators and recruiting (only 1.1% of the all
48 seeds used in the two experiments), indicating that the survival of seeds is a critical step in the life cycle of this plant,
49 highlighting the role of dispersal mode in recruiting success.

50 **Keywords:** seed dispersal patterns, Paraná pine, scatter-hoarding animals, seed predation.

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61 Introduction

62 Animal-plant interactions may suffer the influence of several factors, such as the season, environment
63 characteristics, and the species involved in these interactions, and it can exert both negative (ex. seed predation)
64 and positive (ex. seed dispersal) effects on plants reproduction success (Kersch and Fonseca 2005; Jordano *et al.*
65 2006; Soltani *et al.* 2018; Hernández-Brito *et al.* 2021). These processes are fundamental in the life cycle of plants
66 and ultimately participate in the regulation and structuring of plant communities (Janzen 1971; Schupp *et al.* 2010).
67 While seed predation can induce negative effects on predated plants (Gómez *et al.* 2003; Brocardo *et al.* 2018),
68 seed dispersal is a mandatory process for permanence, regeneration, habitats colonization, and gene flow (Jansen
69 *et al.* 2004, Zhu *et al.* 2019). This way, both are imperative for maintaining plant diversity in tropical forests
70 (Vieira and Iob 2009; Villar *et al.* 2020).

71 According to the Janzen-Connell model the likelihood of seed survival is higher far from conspecific
72 adults, but this model is mainly applied when the predators are invertebrates (Janzen 1970; Connell 1971). The
73 lack of efficient seed dispersal mechanisms can generate competition between plants, affecting the survival and
74 reproduction of individuals (Kula *et al.* 2020). However, even though since seed dispersal has singular importance
75 for the dynamics of the animal and plant community, being a fundamental tool for understanding different levels
76 of biological organization (individual, species, population, and landscape), it still remains as one of the less known
77 biological processes (Musciano *et al.* 2020).

78 Zoochoric dispersal occurs between 50% to 90% of tropical tree species, evidencing animals' importance
79 for plant recruitment (Francisco and Galetti 2001). Among the most singular forms of zoochoric dispersers are the
80 scatter-hoarding animals (synzoochory), which are seed predators that commonly store seeds in branches or under
81 the ground to survive periodic shortages of food (Smith and Reichman 1984; Vander Wall 2001). However, some
82 of the hidden seeds end up not being recovered, presenting high germination rates since they are protected from
83 desiccation, freezing, fire, pests, and predators (Forget 1990; Kollmann and Schill 1996; Jansen and Forget 2001;
84 Gómez 2004; Lichti *et al.* 2017).

85 Scatter-hoarding mammals and birds usually store the seeds near logs, shrubs, and tree trunks (Pons and
86 Pausas 2007; Ribeiro and Vieira 2016), which are likely used as visual landmarks for spatial memory (Barkley *et al.*
87 1998). Also, it is known that when plants are used as reference points, they may serve as nurse plants for seeds
88 and seedlings, offering more favorable micro-habitats to the germination and recruitment of seedlings, facilitating
89 their growth and development (Carlucci *et al.* 2011a; Carlucci *et al.* 2011b; Gómez-Aparicio *et al.* 2008; Padilla
90 and Pugnaire 2006). These plants play a prominent role in the recovery of primary ecosystems, providing nutrients,

91 water, protection from grazing, and trampling of seedlings (Ren *et al.* 2008; Gómez-Aparicio *et al.* 2008; Carlucci
92 *et al.* 2011a), and this process was verified for the Araucaria Forest, whose main element is the Paraná pine
93 (*Araucaria angustifolia*) and has expanded into pasture environments (Hueck 1953; Carlucci *et al.* 2011b).

94 The seed maturation of the Paraná pine occurs primarily in Austral Autumn and Winter (Mattos 2011)
95 and this species is considered a key species, providing food for the regional fauna (Vieira and Iob 2009; Brum *et*
96 *al.* 2010; Brocardo *et al.* 2018; Bogoni *et al.* 2020; Rosa *et al.* 2020). It presents large recalcitrant seeds (> 5 g),
97 locally named “pinhão”, which need zoochoric dispersal to reach new environments (Soares and Mota 2004). And,
98 even though its recruitment is strongly impacted by animals (Iob and Vieira 2008; Brocardo *et al.* 2018) this factor
99 has received little attention in studies on the population dynamics of the species (Klein 1960; Souza *et al.* 2008;
100 Paludo *et al.* 2016). Since the beginning of the 20th century the Paraná pine was heavily exploited for its valuable
101 wood, reducing the current Araucaria Forest cover to only 12% of the original area (Mähler-junior and Larocca
102 2009; Ribeiro *et al.* 2009). Today this species presents low regeneration in forest fragments and has been suffering
103 from deforestation caused by the expansion of agriculture, biological invasions, and a high rate of predation on its
104 seeds (Emer and Fonseca 2011; Brocardo *et al.* 2018).

105 There are two ways that the Paraná pine seed dispersal occurs, the first, when a predator accidentally
106 drops the seed onto the ground during displacement, which can occur with parrots (Tella *et al.* 2016), monkeys
107 (CR Brocardo, obs. pers.), and small rodents (Brum *et al.* 2010; Vieira *et al.* 2011). The second is when scatter-
108 hoarding animals actively bury seeds in the soil as a form of food supply, such as the plush-crested jay (*Cyanocorax*
109 *chrysops*) (Uejima *et al.* 2012) and the green agouti (*Dasyprocta azarae*) (Brocardo *et al.* 2018). However, it is
110 not known yet how the different dispersal modes promoted by the animals and the places where they leave the
111 seeds can affect the successful establishment of the Paraná Pine (Vieira *et al.* 2001; Brum *et al.* 2010; Brocardo *et*
112 *al.* 2018). Tracking the seed's fate until germination or seed decay is a complicated process, as the scatter-hoarding
113 animals actively manage its caches, sometimes relocating the seeds (Jansen and Forget 2001; Vieira and Iob 2009).
114 In consequence, some studies focusing on the Paraná pine's seed dispersal were limited to evaluating only the first
115 path of the predation and removal process, without monitoring the seeds until their final destination (e.g. Iob and
116 Vieira 2008; Pereira and Ganade 2008; Brocardo *et al.* 2018; Brum *et al.* 2010).

117 Studies related to seed dispersal provide necessary information for conservation biology, addressing the
118 different ecological and evolutionary processes (Musciano *et al.* 2020). The effectiveness of a disperser animal
119 species is directly linked to the contribution generated to the plant's future reproduction and is related to the number
120 of dispersed seeds and the quality of this dispersal (Schupp 1993; Schupp *et al.* 2017). However, the qualitative

121 aspects of the contributions of each disperser animal species and its effect on different environmental
122 characteristics remain unclear for several species and ecosystems (Schupp 1993; Gómez 2004).

123 Acknowledging the importance of dispersal aspects for several critical ecology issues (Schupp *et al.*
124 2010), besides the fact that the Paraná pine is a threatened species (Thomas 2013), we aimed to evaluate the effect
125 of seed dispersal mode (according to the disperser), the distance from adult conspecifics and the disposition site
126 on recruitment success of Paraná pine. Our hypotheses were: Hypothesis 1. Scatter-hoarding is the most effective
127 dispersal mode to establish *Araucaria angustifolia*. We predict that buried seeds have superior survival and
128 germination rates when compared to seeds left above the ground. Hypothesis 2. The distant dispersal from
129 conspecific adults is more important in open environments than in forest environments. We predict that the distance
130 from conspecific adults does not follow the Janzen-Connell model of seed survival in a forest environment, due to
131 the homogeneous presence of fauna inside the forest, however, in open areas, the predation rate should be highest
132 near conspecific adults, due to the concentration of predators in these areas; Hypothesis 3. Nurse plants have a
133 positive effect on the establishment of *Araucaria angustifolia*, especially in open environments. We predict that
134 nurse plants positively influence seed germination by protecting the seed against dehydration.

135 **Material and methods**

136 *Study area*

137 The study was carried out in the Paulo Gorski Natural Municipality Park (24°57'46.54"S, 53°26'24.76"W)
138 and Danilo Galafassi Natural Municipality Park (24°57'2"S, 53°25'52"W), located in Cascavel, Paraná state, Brazil
139 (24° 57'21 "S, 53° 27'19" W), with an altitude of 730-780 meters above sea level, with a subtropical climate,
140 characterized by the lack of a dry season and mean annual temperature of 19 to 21°C (Castella and Britez 2004)
141 (Fig. 1). The study areas maintain together 110 ha of Araucaria Forest, where species responsible for most
142 interactions with the Paraná pine seeds are found, such as the plush-crested jay (*Cyanocorax chrysops*), the black
143 capuchin monkey (*Sapajus nigritus*), agouti (*Dasyprocta azarae*), rats (family Cricetidae) and parrots (family
144 Psittacidae) (Pagno *et al.* 2015; Brocardo 2017; Brocardo *et al.* 2018).

145

146 *Experiment design*

147 *Effect of the environment, micro-habitat, and dispersal type on seed survival*

148 We simulated dispersal conditions that might occur naturally according to the environment (forest or open
149 field), micro-habitats of dispersal disposition, and type of treatment given by the disperser animal, so we had the
150 following experimental design (Fig. 2):

151 *Environmental disposition*

- 152 • Forest (FT): in this environment, the seeds are dispersed both by scatter-hoarding animals
153 (squirrels, agouti, jays) and by predators (monkeys, rats, parrots) (Brocardo *et al.* 2018).
- 154 • Open field (OF): environment outside the forest, where dispersal occurs mainly by jays and
155 parrots, which use non-forest environments (Uejima *et al.* 2012; Tella *et al.* 2016).

156 *Dispersal micro-habitat*

- 157 • Under conspecific adult (UC): In this micro-habitat seeds left on the ground represent both the
158 direct fall of the seeds and the action of animals foraging in the Paraná pine cones, causing the
159 fall of seeds. Buried seeds below adults represent seeds stored at short distances (Jansen *et al.*
160 2004; Donatti *et al.* 2009) (see next section “Dispersal type done by disperser animal”).
- 161 • Far from conspecific adults and next to a landmark (NL): this site attempts to simulate seed
162 placement preference locations by scatter-hoarding animals, who use landmarks to resemble the
163 cache location (Jansen 2003). We use trees other than the Paraná pine as reference. This disposal
164 site, especially in the open field environment, can influence the success of seedling establishment
165 since it provides a humid micro-habitat (Carlucci *et al.* 2011a). We used 30 m of distance from
166 any female pine, considering the density and grouping of Paraná pine trees in the area (Brocardo
167 *et al.* 2018).
- 168 • Far from conspecific adults and without a landmark (WL): seeds that have been carried a distance
169 by predators or buried by scatter-hoarding animals. In this case, the seeds were made available
170 at a minimum distance of 30 m from any female Paraná pine, considering the density and
171 grouping of Paraná pine trees (Brocardo *et al.* 2018).

172 *Dispersal types done by the disperser animal*

- 173 • Intact seed over the ground (IS): simulating the dispersal by predators (monkeys, parrots, and
174 rats) when they lose or abandon the entire seed during displacement (Job and Vieira 2008; Vieira
175 *et al.* 2011; Tella *et al.* 2016).
- 176 • Partially preyed seed over the ground (PS): simulating the dispersal by predators (rats and
177 parrots), but in this case, the animal had already started consumption when the seed was
178 abandoned (Vieira and Job 2009; Tella *et al.* 2016). In this experiment, part of the seed was
179 removed with a knife (30%), not damaging the embryo, to allow the monitoring of germination
180 (primary root tissue) and establishment of the seedling.

- 181 • Intact buried seed (IB): at a depth of 5 cm simulating the dispersion made by scatter-hoarding
182 animals (agouti, jay, and squirrel) (Brocardo *et al.* 2018).

183 In each environment (FT and OP) 30 sampling stations were established, 10 stations for each dispersal
184 micro-habitat (UC, NL, and WL) (a list of abbreviations used in the text is available in the supplementary material,
185 Table S5). The stations were located at least 30 meters from the other, a distance considered sufficient for sampling
186 independence in seed removal studies (Chauvet and Forget 2005; Sanguinetti and Krizberger 2010). Each of them
187 received five seeds of each dispersal type (IS, OS, IB), totaling 15 seeds per micro-habitat station, 450 per
188 environment, summing 900 seeds in this experiment (2 environments x 30 micro-habitat sampling stations x 3
189 treatments of dispersal type x 5 seeds = 900 seeds).

190 *Seed fate experiment*

191 Since the removal of seeds is frequently done over long distances, locating them becomes a difficult task,
192 making it impossible to verify their fate. Thus, we conducted a parallel experiment in the forest environment,
193 where we marked the seeds with the spool and line technique. This technique consists of attaching a spool of thread
194 to each seed, which is drilled in the distal portion, not interfering with the germination probability (Donatti *et al.*
195 2009). A 20 cm steel line was passed through the perforations to prevent the animals from cutting the sewing line
196 and subsequently connected (SI, Fig. S1, and S2). We used 90 m spools (100% polyester thread), kept inside
197 plastic jars (toothpick holder) to allow unwinding (SI, Fig. S1, and S2). To prevent dissection and fungi attack
198 through the perforations, we used non-toxic white glue for sealing (SI, Fig. S1). It was not possible to repeat this
199 experiment in the open field environment used in this survey since it has an elevated anthropic influence, with
200 daily and constant use by people, which could cause the removal of lines or accidents.

201 In this experiment, the seeds were distributed in the same microhabitats (UC, WL, and NL) and with the
202 same dispersal type treatments (IS, PS, and IB), with the same number of replicates (five seeds in each), totaling
203 450 seeds. Thus, in our study, in total, 1350 Paraná pine seeds were used, being consistent with other studies that
204 tested the effects of microhabitat and burial on the seed predation rates, such as studies with *Quercus ilex* and
205 *Quercus pyrenaica* (1000 and 1600 seeds, respectively) (Gómez *et al.* 2003; Gómez 2004).

206 The seeds were selected using the floating technique (floating seeds were discarded) and by the visual
207 inspection (larvae infestation) to attest germination viability (Doni Filho *et al.* 1985). We placed the seeds in the
208 field in June 2019, during the maturation period of the Paraná pine seeds (autumn-winter), and we monitored them
209 every 30 days to verify predation, removal, and recruitment. Follow-up continued for 120 days, when, probably,
210 most of the seeds should have already germinated (Soares and Mota 2004). The identification of species that

211 interact with the seeds in the second experiment was based on marks left by animals on the seeds after predation
212 (Müller 1986; Squinzani *et al.* 2021).

213 *Statistical Analysis*

214 We used the classic analysis of variance with repeated measures as statistical inference (RM-ANOVA),
215 followed by the corrective procedures by Geisser and Greenhouse (1958) and Huynh and Feldt (1976), GG and
216 HF, respectively, for sphericity. As the survival data were measured in proportions of the initial value, the
217 transformation into arcsine of the square root was used, we also checked the conditions of normality and
218 homogeneity of variance in the residuals of the statistical model (Queen *et al.* 2002). With repeated measures
219 analysis of variance, it was possible to verify and compare results of different trials over time, analyzing the same
220 experimental unit over and over again (*sensu* Nobre and Singer 2007).

221 In the trial to evaluate the effects of the environment, micro-habitat, and type of dispersion, on the survival
222 of unmarked seeds, the complete three-factor RM-ANOVA was used for interactions up to third order. In the assay
223 with marked and unmarked seeds in the different treatments, survival was evaluated with complete two-factor RM-
224 ANOVA for interactions up to second order. The latter was also used to evaluate the effects of the types of
225 dispersers and micro-habitat on the survival of marked seeds.

226 **Results**

227 *Effects of the environment, micro-habitat, and dispersal type*

228 In the first experiment (unmarked seeds), from 900 seeds used to assess the effects of the environment,
229 micro-habitat, and dispersal type, 11.8% were predated on the initial position site (FT=11.5% in and OP=12%),
230 possibly by plush-crested jay (*Cyanocorax chrysops*), green agouti (*Dasyprocta azarae*), capuchin monkey
231 (*Sapajus nigritus*) and small rodents, based on the marks left on Paraná pine seeds (Squinzani *et al.* 2021) (SI –
232 Fig. S3). However, most of the seeds were removed from the site, corresponding to 87.3% of the total (FT=88.4%
233 and OF=86.2%), 0.33% of the them remained intact without germinating until the last survey (FT = 0% and OF =
234 0.66%), 0.11% became unviable due to decay (FT = 0% and OF = 0.22%) and only 0.44% germinated until the
235 last survey (FT =0% and OF=0.88%). Although the final survival rate was higher in the open field (OF), the
236 environment was not significant (F=0.37, p=0.53).

237 The micro-habitat (seed dispersal site), as well as the environment, did not explain the seed survival alone
238 (F=0.67, p=0.51). On the other hand, the dispersal type provided by the disperser was significant for survival
239 (F=10.87, p<0.001), and the buried seeds were the only ones that survived until the last survey (Fig. 3). In the open
240 field (OF) area, buried seeds (IB) far from conspecific adults and next to a landmark (NL) (n=50) presented 8% of

241 germination, which is equivalent to 0.88% of all seeds in this environment (n=450), or 0.44% of the total (n=900).
 242 Still, 4% of OF-NL-IB seeds (initial n=50) and 2% of OF-UC-IB (n=50) remained possibly viable, but they did
 243 not germinate, totaling 0.66% of the seeds in open fields (n=450), or 0.33% of all seeds (n=900). This resulted in
 244 a significant interaction between environment and micro-habitat ($F=3.78$, $p=0.02$), indicating that seeds in open
 245 fields, far from conspecific adults, and next to landmarks are more likely to survive.

246 The highest animal-seed interaction rates occurred in the first survey, after 30 days of initial disposal,
 247 with 89.5% of the total, when the largest number of seeds were removed and predated, both in the open field (OF
 248 = 88.6%) and in forest (FT=90.6%), which resulted in a significant response for the time factor (Table 1: $F=20.63$,
 249 $p<0.001$, $p[GG]<0.001$, $p[HF]<0.001$). The interaction of time factor and dispersal type was also significant
 250 ($F=8.13$, $p<0.001$, $p[GG]=0.08$, $p[HF]<0.001$), indicating that buried seeds (IB) survived for a longer time (SI,
 251 Fig. S4); as well as the interaction time, environment and dispersal type, which was significant ($F=4.84$, $p<0.001$,
 252 $p[GG]<0.01$, $p[HF]<0.004$), driven by the higher survival of buried seeds (IB) in the open field (OF) (Fig. 3ab).
 253 Finally, significance was observed in the triple interaction between the environment, micro-habitat, and time
 254 ($F=3.62$, $p=0.001$, $p[GG]=0.01$, $p[HF]=0.01$), in which the seeds disposed in the open field (OF), far from
 255 conspecific adults and next to landmark (NL), and buried (IB), survived for a longer period (Fig. 3cd).

256 *Seed fate experiment*

257 The information obtained from the seeds marked in the forest concerning the dispersal types corroborates
 258 with the results obtained from the test with unmarked seeds, as a significant effect of the interaction between the
 259 dispersal type and time was observed (RM_ANOVA: $F=12.71$, $p<0.0001$, $p(GG)<0.0001$, $p(HF)<0.0001$), in
 260 which intact buried seed (IB) survived longer (Fig. 5), regardless of micro-habitat (RM_ANOVA, micro-
 261 habitat*time: $F= 1.01$, $p= 0.42$, $p(GG)0.40$, $p(HF)= 0.40$).

262 The Paraná pine seeds marked with spool and line followed the same pattern as the unmarked seeds,
 263 having the highest interaction rates with animals during the first survey (30 days after the placement of the seeds).
 264 In this phase, 64.9% of seeds were predated, while 8.8% were stocked, 14% had not been removed from the
 265 disposal sites, and 12.2% were removed, but due to line breakage it was not possible to reach the destination. Up
 266 to the last survey 81.5% of the seeds had been predated and 14.6% were removed but lost due to broken lines (Fig.
 267 4).

268 There was only one secondary storage in the 60-day survey, without storage in the other reads and,
 269 therefore, the number of stored seeds did not change from the first survey to the others. The average distance of
 270 the storage locations in relation to the initial position of the seeds was only 2.81 m, ranging from zero (buried in

271 the initial position) to 13.8 m.

272 Intact buried seeds (IB) in their initial position were the only ones to germinate, that occurred in the third
 273 and fourth surveys (90 and 120 days respectively), representing 1.33% of the total, although just 0.88% survived
 274 until the end of the experiment (SI, Table S4). Possibly viable Paraná pine seeds that had not germinated yet totaled
 275 1.77%, mostly corresponding to intact buried seeds (IB= 1.54%), and only one seed (0.22%) removed and stored
 276 by an animal. Some seeds also had mortality linked to other causes, such as dehydration and rot, totaling 1.11%.

277 From the predated seeds the main responsables were the Plush-crested jays (*Cyanocorax chrysops*)
 278 (39.56% of the seeds), small rodents (22.67%), the green agouti (*Dasyprocta azarae*) (16%), and insects attacked
 279 1.33% of seeds (SI, Fig. S3).

280 When comparing marked and unmarked seeds in the forest environment (FT) no interactive effect of
 281 marking was identified over the experimental days (RM-ANOVA: $F(3, 24) = 1.62, p = 0.21$), but we observed an
 282 effect for marking (RM-ANOVA: $F(1, 8) = 11.89, p = 0.009$), in which the spool and line technique obtained
 283 higher survival at all times sampled. Unmarked Paraná pine seeds were preferentially selected by the animals,
 284 decreasing their survival compared to labeled seeds from the first reading to 30 days (Fig. 6). The interaction
 285 between treatments and time was also significant (RM-ANOVA: $F(24, 24) = 3.02, p = 0.004$; GG: $e = 0.4146, p$
 286 $= 0.048$; HF: $e = 0.4567, p = 0.040$), with faster removal of seeds left over the ground, especially in relation to PS
 287 that were all removed and preyed on the first reading (95% predated and 5% removed without destination
 288 registration).

289 Discussion

290 The removal rate of Paraná pine seeds by animals was high in both experiments and the marking
 291 experiment attested that most of the seeds suffered predation after removal, which was probably the same fate of
 292 seeds removed in the first experiment. This result can be linked to several factors, such as the attractiveness of
 293 large seeds with high energetic value and their maturation in a food scarcity period, serving as a key resource for
 294 fauna during Winter (Vieira and Iob 2009; Brocardo et al. 2018; Bogoni et al. 2020; Rosa et al. 2020). The very
 295 high seed predation rate that we have recorded, with only 1.1% of the seeds reaching germination, corroborates
 296 that the seed survival from predators is a critical stage in the life cycle of the Paraná pine (Paludo et al. 2016,
 297 Brocardo et al. 2018; Rosa et al. 2020), highlighting the role of dispersal type in the recruiting success.

298 In this sense, storage was the most successful type of dispersal, validating hypothesis I, with buried seeds
 299 presenting higher survival and germination rates for *A. angustifolia*. This result can be explained by the protection
 300 that buried seeds have against predators (Gómez 2004; Lichti et al. 2017; Brocardo et al. 2018; Martínez-Baroja

301 et al. 2021) and by the better moisture conditions (Forget 1990; Kollmann and Schill 1996), demonstrating the
302 importance of the relationship between scatter-hoarding animals and the Paraná pine. Despite that, the spool and
303 line experiment revealed that only 0.2% of the seeds stored by animals (9,1%) survived to secondary dispersal
304 during the experiment. Overall, plants that are dispersed by scatter-hoarding animals suffer high seed predation
305 rates, as evidenced for *Bertholletia excelsa*, with scatter-hoarding rodent recovery up to 99.4% of cached seeds
306 (Haugaasen et al. 2010). In another example, for *Carapa procera*, a tropical tree that is also dispersed by scatter-
307 hoarding animals, the establishment of seedlings was less than 1% of the seeds available to the animals, ranging
308 from 0.7%, in a year of high seed production, to only 0.1% in low production years (Jansen et al. 2004). Most
309 scatter-hoarding animals are well suited for retrieving their hiding places relying on spatial memory and olfactory
310 cues (Vander Wall 2000), estimates of scatter-hoarding retrieval by squirrels and other small mammals often
311 exceed 90% (Steele et al. 2005). Additionally, environmental conditions can determine the quantity and quality of
312 seed storage and this can be decisive for the recovery (Jansen and Forget 2001). The high olfactory capacity of
313 rodents increases the chances to recover cached seeds, being these post-dispersal interactions a critical stage to the
314 recruitment effectiveness of nut-producing trees (Haugaasen et al. 2010; Elwood et al. 2017).

315 On the other hand, seeds left above the ground did not present survival. Partially predated seeds,
316 simulating dispersal provided by rats and parrots (Tella et al. 2016), were the first to be predated and removed. In
317 the experiment with marked seeds, it was possible to verify that almost all of them were preyed during the first
318 survey (30 days). These results indicated that parrots and rats partially prey on seeds and abandon them, and even
319 if they do not affect the embryo, have little efficiency as dispersers. Entire Paraná pine seeds left above the ground,
320 simulating the dispersal by predators (monkeys, parrots, and rats) when they lose or abandon the entire seed during
321 movement (Iob and Vieira 2008; Vieira et al. 2011; Tella et al. 2016), also had little success, probably due to the
322 exposure to predators.

323 The micro-habitat, which represented sites where seeds may be dispersed, did not explain survival alone,
324 although it was significant in interaction with the environment, with higher seed survival rates far from conspecific
325 adults and next to a landmark in the open field, corroborating with the hypothesis 2. As expected, the distance
326 from conspecific adults did not result in higher survival rates in FT, due to the homogeneous presence of the fauna
327 (Brocardo et al. 2018), indicating that predation did not follow the Janzen-Connell model, which postulates a
328 higher chance of seed survival far from conspecific adults (Janzen 1970; Connell 1971). The UC microhabitat
329 presented longer survival than the other microhabitats at the 30-day survey, a similar pattern observed for *Virola*
330 *michelii* in French Guiana (Forget et al. 2000). In this case, in a forest environment, the greater availability of seeds

331 below reproductive adults can generate more satiety to predators, delaying seed removal (Vander Wall 2002; Job
332 and Vieira 2008; Xiao et al. 2013). The opposite result was observed in OF, where the predation rate was superior
333 under conspecific (UC), probably in response to the concentration of predators in these areas (Gómez et al. 2003;
334 Gómez 2004; Sanguinetti and Kitzberger 2009).

335 In the OF, the greatest survival was observed in seeds far from conspecific adult next to landmark,
336 corroborating hypothesis 3. This form of storage in an OF in our study environment reflects the behavior of
337 *Cyanocorax chrysops*, which as other corvids use landmarks as a reference to spatial memory (Balda and Kamil
338 2006). Landmark trees then function as nurse plants providing a favorable environment for germination (Gómez-
339 Aparicio et al. 2008; Carlucci et al. 2011b), which may contribute to Paraná pine expansion in open areas. The
340 expansion of *A. angustifolia* over grasslands has been seen as characteristic of a restrict and pioneer heliophile
341 species (Klein 1960), although young plants tolerate shading (Duarte and Dillenburg 2000; Duarte et al. 2002;
342 Sanguinetti 2014; Brocardo et al. 2018), indicating light demand is not responsible to the low recruitment in the
343 forest. Our results indicate the high regeneration in open and border areas could be associated with higher seed
344 survival, due to the lower abundance of predators, corroborating previous studies (Lamberts 2003; Brum et al.
345 2010). Thus, expansion over open areas would reflect an escape from predation rather than a response to the
346 demand for light.

347 **Conclusion**

348 For the Paraná pine, which relies its seed dispersal on seed predators, the success of establishment is
349 strictly linked to the way and location where the seeds are placed, and their density in the environment. Among
350 the animals that can disperse Paraná pine seeds, scatter-hoarding animals are probably more effective for survival
351 and germination of seeds, as they cache the seeds, decreasing the chances that other animals found them, in
352 addition, cached seeds have better conditions to recruit. In the open field the micro-habitat of seed deposition can
353 also influence the germination efficiency, especially those that have the action of nurse plants. The processes of
354 predation and seed dispersal of Paraná pine are largely responsible for the successful recruitment of the species,
355 being probably one of the most important phases of its life cycle, which should be taken into account in recovery
356 plans and conservation of the Araucaria Forest.

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569 **Table 1** Results of classical repeated-measure of three-factor analysis of variance for Paraná pine seed survival
 570 (data transformed into arcsine square root). The test statistics (ϵ) and probabilities obtained by the corrective
 571 procedures for sphericity of Geisser and Greenhouse (1958) and Huynh and Feldt (1976), p(GG) and p(HF),
 572 respectively, are presented. Values in bold indicate significant effects ($p < 0.05$). For the effects involving time,
 573 only those significant in the three tests (p, p(GG) and p(HF)) were interpreted.

Explanatory variable			$\epsilon = 0.440$;	$\epsilon = 0.442$;
	F	p	p(GG)	p(HF)
Environment	0.3794	0.5388		
Micro-habitat	0.6709	0.5127		
Dispersal	10.8722	<0.0001		
Environment: Micro-habitat	3.7825	0.0248		
Environment: Dispersal	0.2219	0.8013		
Micro-habitat: Dispersal	0.4976	0.7375		
Environment: Micro-habitat: Dispersal	1.9234	0.1090		
Time	20.6329	<0.0001	0.0008	<0.0001
Environment: Time	0.2446	0.8652	0.6881	0.6893
Micro-habitat: Time	0.1845	0.9811	0.8856	0.8864
Dispersal: Time	8.1390	<0.0001	0.0851	0.0001
Environment: Micro-habitat: Time	3.6255	0.0016	0.0179	0.0177
Environment: Dispersal: Time	4.8440	0.0001	0.0042	0.0041
Micro-habitat: Dispersal: Time	0.6650	0.7854	0.6587	0.6595
Environment: Micro-habitat: Dispersal: Time	1.2743	0.2302	0.2745	0.2744

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581 List of Figures

582 **Fig. 1** Location of Cascavel in Paraná state, Brazil, highlighting the study areas: Danilo Galafassi Natural
583 Municipality Park “A”, Paulo Gorski Natural Municipality Park “B” and their corresponding sampling stations.
584 UC: under conspecific adults, NL: far from conspecific adults and next to landmark, WL far from adults and
585 without landmark.

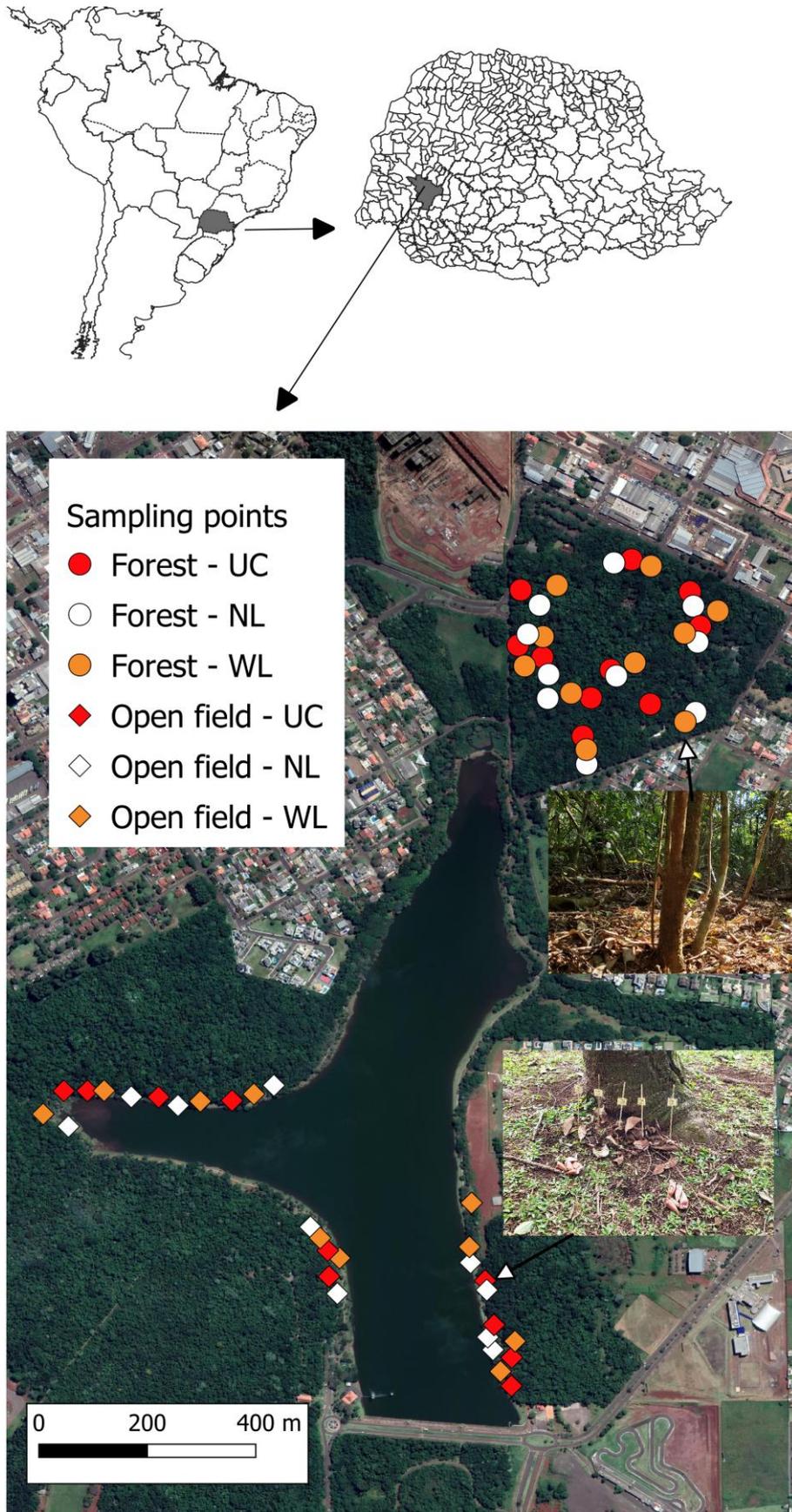
586 **Fig. 2** Representation of the experimental design that has eighteen treatments, formed by the factorial arrangement
587 of two disposition environments (FT and OF) with three types of micro-habitat sites (UC, WL, and NL) and three
588 dispersal types (PS, IS, and IB). Data collection contained four replicates of 30 days, 60 days, 90 days, and 120
589 days.

590 **Fig. 3** Survival (proportion of non-predated and possibly viable seeds) of Paraná pine seeds, categorized by
591 dispersal types (IB= intact buried seed; IS= intact seed over the ground; PS= partially preyed seed over the ground),
592 observed in the open field (a) and in the forest (b); and categorized by micro-habitats of dispersion (UC= under
593 conspecific adult; NL= far from conspecific adults and next to landmark; WL= far from conspecific adult and
594 without landmark), observed in the open field (c) and in the forest (d). Bars indicate standard error, estimated on
595 data transformed to arcsine square root and converted back to proportion. In it, it is possible to verify differences
596 in survival, being more effective the open environment with the micro-habitat far from conspecific adults and next
597 to landmark and the intact buried seed treatment.

598 **Fig. 4** Destination of seeds marked in FT environment in the dispersal types (PS, IS and IB) in 3 types of micro-
599 habitat (UC, WL and NL). The sampling contains 4 replicates of 30, 60, 90 and 120 days.

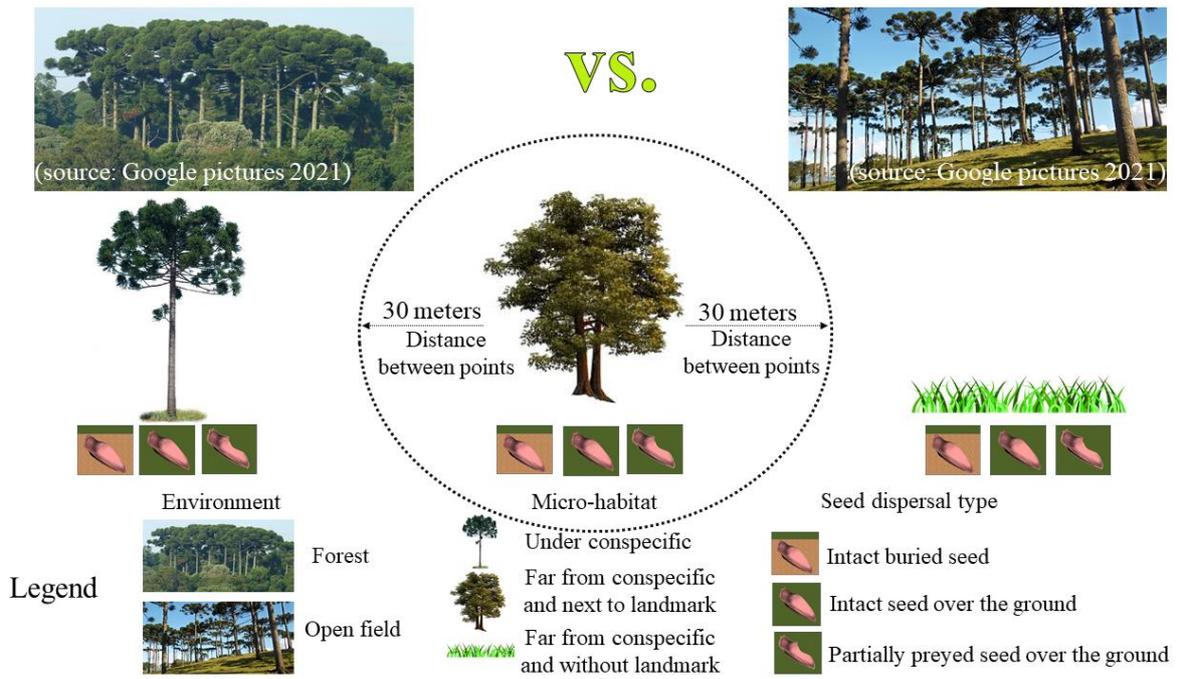
600 **Fig. 5** Survival (proportion of non-predated and possibly viable seeds) of Paraná pine seeds in relation to types of
601 dispersion (IB = intact buried seed; IS = intact seed over the ground; PS = partially preyed seed over the ground)
602 throughout the experimental days. Bars indicate standard error, estimated on data transformed to arcsine square
603 root and converted back to proportion. IB was more effective in the survival of the seeds.

604 **Fig. 6** Survival (proportion of non-predated and possibly viable seeds) of Paraná pine seeds with and without
605 marks for tracking throughout the experimental days. Bars indicate standard error, estimated on data transformed
606 to arcsine square root and converted back to proportion. Seeds with marking had greater survival at all times
607 sampled.



608

609 Fig. 1



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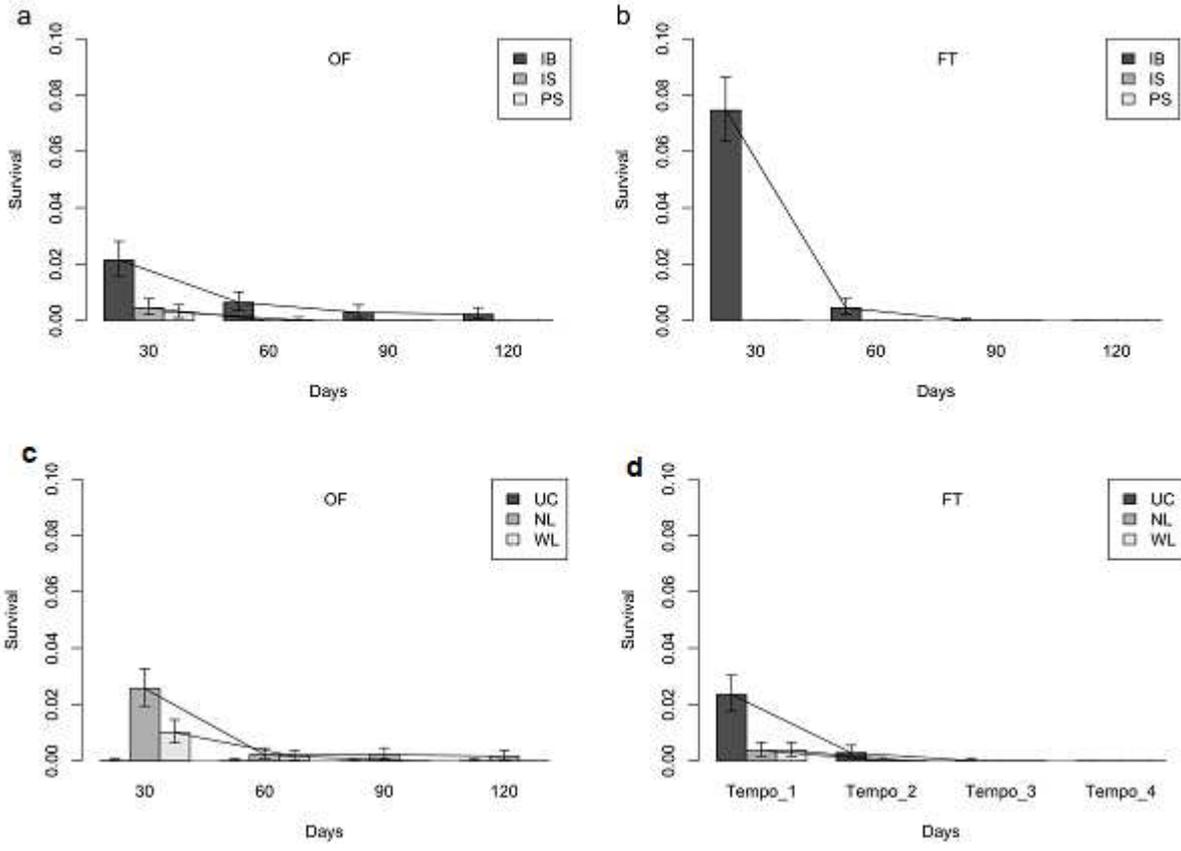
611 **Fig. 2**

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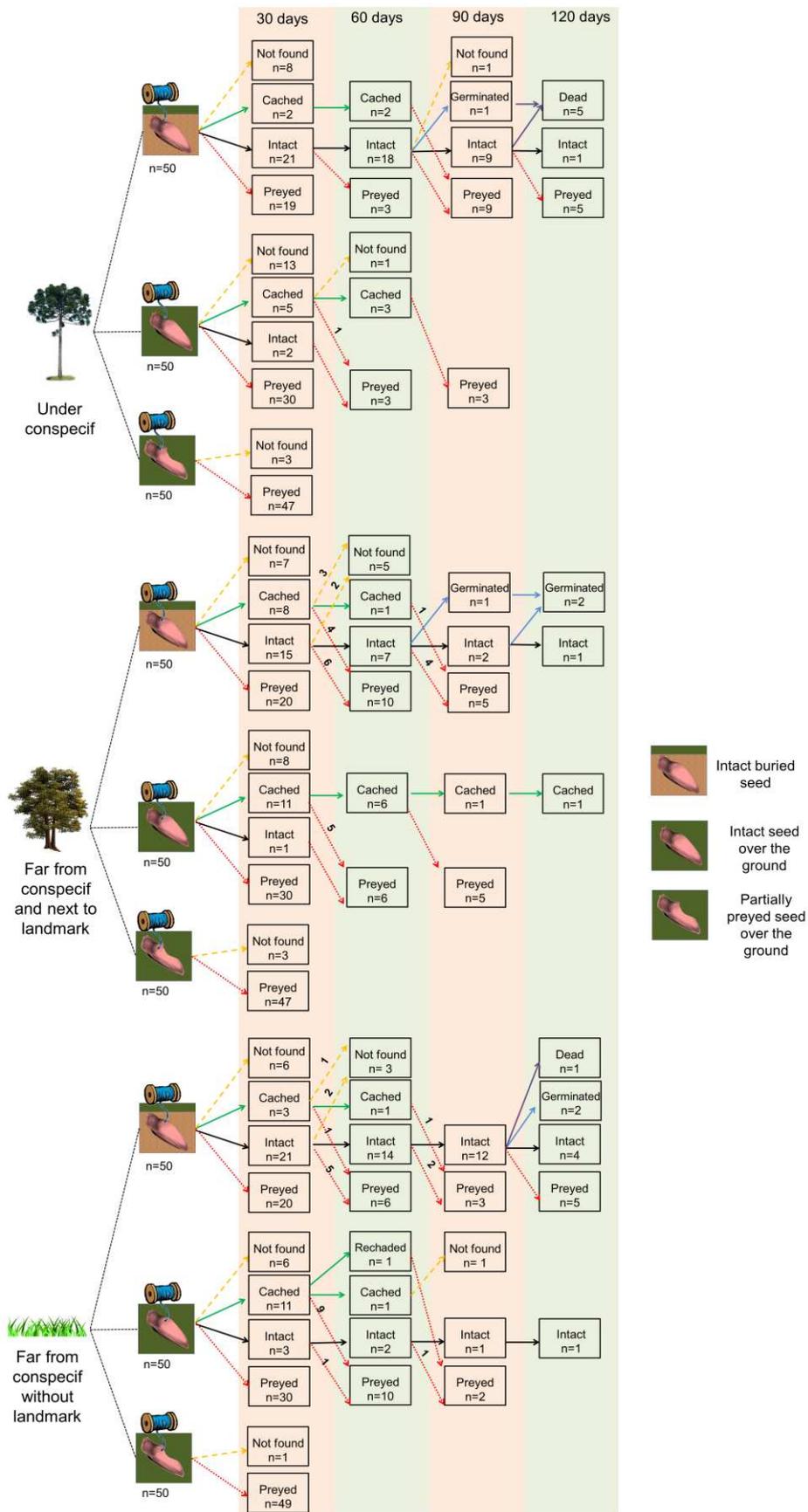
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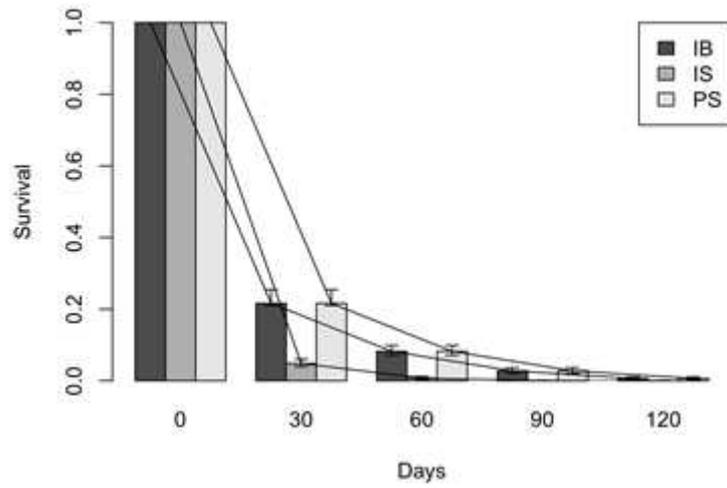
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617 Fig. 3



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619 Fig. 4



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621 Fig. 5

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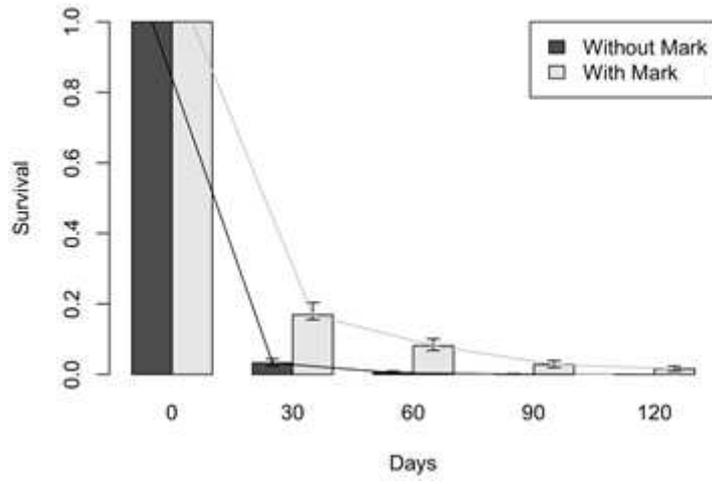
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634 **Fig. 6**

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Supplementary Files

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