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Effect of Fruit Handling by Frugivores on Seed Dispersal Effectiveness

RAÍSSA SEPULVIDA

ORIENTADORA: LAURENCE M. V. CULOT

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Dissertação apresentada ao Instituto de Biociências do Câmpus de Rio Claro, da Universidade Estadual Paulista, como parte dos requisitos para obtenção do título de mestre em Ecologia e Biodiversidade.

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RESUMO

A dispersão de sementes por animais contribui para manutenção da biodiversidade em florestas tropicais. A zoocoria é um processo importante para muitas espécies de plantas porque diminui a lacuna entre a produção de frutos e o recrutamento de plântulas. A manipulação das sementes por frugívoros (através da limpeza das sementes e remoção destas para longe do coespecífico) contribui para reduzir a mortalidade de sementes e aumentar o sucesso de germinação e recrutamento. No entanto, ainda pouco se sabe o quanto esses efeitos variam de acordo com as características dos frutos/sementes. Estudos sugerem que a limpeza da semente afeta positivamente espécies com frutos de polpa carnosa, pois a remoção da polpa reduz a atração de patógenos. A remoção da semente para longe das árvores coespecíficas auxilia as sementes a escaparem da mortalidade dependente da densidade. Esse efeito, portanto, deve ser maior em espécies submetidas a um forte *feedback* negativo da relação planta-solo. Nesse estudo, nós investigamos como a limpeza da semente e a remoção para longe do coespecífico afetam a germinação, emergência de plântulas e sobrevivência das sementes de três espécies que possuem características distintas: *Castilla ulei* (Moraceae; polpa carnosa com alta aderência e sementes com tegumento fino), *Hymenaea parvifolia* (Fabaceae; vagem lenhosa resistente, polpa farinácea e tegumento espesso) e *Byrsonima arthropoda* (Malpighiaceae; polpa carnosa revestindo um pirênio lenhoso e resistente que reveste 2-3 sementes). Nós também estimamos a variação percentual do efeito do manuseio pelo frugívoro na eficácia da dispersão de sementes (EDS – estimado como a proporção de sementes/plântulas que sobrevivem até o final do estudo) das plantas quando comparado ao sucesso reprodutivo esperado na ausência dos dispersores. Para isso, nós realizamos um experimento de campo no sudeste da Floresta Amazônica (terra firme), Mato Grosso, aplicando uma combinação de tratamentos às sementes das espécies: sementes limpas, sementes com polpa, debaixo ou longe de uma árvore coespecífica. A limpeza da semente aumentou significativamente a germinação, a emergência de plântulas e a sobrevivência da *C. ulei* enquanto a remoção das sementes para longe do coespecífico teve um efeito negativo, porém fraco, na emergência de plântulas. Nenhuma semente com polpa dessa espécie sobreviveu, assim, a limpeza das sementes por frugívoros aumentou de forma assintótica o EDS de *C. ulei*. A deposição de sementes embaixo do heterospecífico aumentou significativamente o sucesso de germinação de *H. parvifolia*, porém, de forma fraca. A limpeza da semente e a remoção não afetaram a emergência de plântulas nem a sobrevivência. Apesar do efeito positivo (porém, fraco) da remoção da semente para longe do coespecífico na germinação da *H. parvifolia*, tal efeito não foi suficiente para aumentar a sobrevivência de sementes/plântulas, a qual, na verdade, decresceu por até 25%. *B. arthropoda* não germinou durante o período de estudo e nós encontramos que a limpeza da semente diminuiu a sobrevivência da espécie, consequentemente reduzindo o EDS por até 75%. Nossos resultados sugerem que a limpeza da semente e remoção para longe do coespecífico afetam as plantas de forma diferente de acordo com o estágio de vida e com as espécies. Essa diferença na resposta pode ser explicada pelas características dos frutos e sementes. Pesquisas adicionais investigando as respostas das plantas ao manuseio pelo frugívoro de acordo com característica funcionais irá auxiliar a prever o efeito da ausência de dispersores de sementes na regeneração das florestas.

Palavras-chave: dispersão de sementes, manipulação de frutos, Janzen-Connell, floresta Amazônica.

ABSTRACT

Seed dispersal by animals is an important process for the maintenance of biodiversity in tropical forests. Zoochory is of great importance for many plant species as it increases seedling recruitment probability. Seed handling by frugivores (seed cleaning and removal away from conspecifics) has the potential to reduce seed mortality and increase germination and recruitment success. However, it is still unknown how much these effects vary according to fruit/seed traits. Indeed, we can expect that seed cleaning affects more positively fleshy-pulp fruits since this treatment reduces the pathogen infestation that is more likely to occur in such fruits. Seed removal from conspecific trees helps to escape the density-dependent mortality and its effect might thus be stronger for species suffering high negative plant-soil feedback. Here, we investigated how seed cleaning and removal away from conspecifics affect the germination, seedling emergence and survival of three species with distinct traits: *Castilla ulei* (Moraceae; fleshy pulp hardly adhered to the soft seed), *Hymenaea parvifolia* (Fabaceae; hard-wood pod, farinaceous pulp and hard seed) and *Byrsonima arthropoda* (Malpighiaceae; fleshy pulp around a hard-wood pyrene with 2-3 seeds). We also estimated how much the seed handling by frugivores changes the seed dispersal effectiveness (SDE - estimated as the proportion of seed/seedling surviving until the end of the study) of plants compared to the expected reproductive success in absence of seed dispersers. We conducted a field experiment in the southern Brazilian Amazon (*terra firme* forest), Mato Grosso, applying the combination of treatments to seed species: cleaned seeds, seeds with pulp, under and away conspecific trees. Seed cleaning significantly increased the germination, seedling emergence success and had a weak positive effect on survival of *C. ulei* while removal away from conspecifics had only a weak negative effect on seedling emergence. Since none of undispersed seeds survived, seed cleaning by frugivores increased *C. ulei* SDE asymptotically. Seed deposition under heterospecific trees significantly increased the germination success of *H. parvifolia*, but weakly. Neither removal nor seed cleaning affected seedling emergence and survival. Despite the positive (but weak) effect of seed removal away from conspecifics on *H. parvifolia* germination, it was not sufficient to positively affect seed/seedling survival, which was actually decreased by up to 25%. *B. arthropoda* did not germinate during the study period, and we found that seed cleaning decreased seed survival, consequently decreasing the SDE by up to 75%. Our results suggest that seed handling and removal from conspecifics affect the plants differently according to the life stages and the plant species. Such discrepancy may be explained by the traits of fruits and seeds and further research involving plant responses to seed handling according to functional traits would help to better predict the effect of the absence of dispersers on forest regeneration.

Keywords: seed dispersal, fruit handling, Janzen-Connell, Amazon forest.

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1. INTRODUCTION

Seed dispersal by vertebrates is an important process for the maintenance of biodiversity in tropical forests (HOWE; SMALLWOOD, 1982; STEVENSON, 2011a). In tropical rainforest, approximately 90% of plant species rely on vertebrates to disperse their seeds (JORDANO, 2000). The zoochoric dispersal is a mutualistic interaction in which the animals benefit from consuming the fruit nutrients and water and the plants gain with the dispersal of their seeds to suitable places for establishment (HOWE; SMALLWOOD, 1982). Ultimately, the seeds will be effectively dispersed if the frugivore activity not only moves them away but contributes to increasing the plant fitness (SCHUPP, 1993). Seed cleaning and removal away from conspecifics are two aspects of seed handling by frugivores that can enhance seed germination and survival. Indeed, a rich literature indicates that the escape from the vicinity of parent tree increases germination and survival probability (COMITA et al., 2014) while other studies show that seed cleaning is more determinant to enhance germination success than the removal away from conspecific trees (FRICKE et al., 2013; LEVI; PERES, 2013). However, we still know little about how the effect of both treatments varies according to plant species and whether it depends on specific fruit and seed traits.

The 'seed dispersal effectiveness (SDE)' concept, as revisited by Schupp et al. (2010), helps to understand how dispersal contributes to the overall plant recruitment and is estimated through a quality and a quantity components. Such concept can be viewed as the "dispersal effectiveness a plant receives" from its multiple agents (SCHUPP; JORDANO; GÓMEZ, 2010a). An effective dispersal enables the plants to overcome a critical bottleneck between seed and seedling recruitment (CHAMBERS; MACMAHON, 1994). The benefits plants receive from dispersal are multiple, such as colonizing new habitats, reaching suitable microsites (HOWE; SMALLWOOD, 1982), tracking climate and environmental changes (IBÁNEZ et al., 2006), promoting gene flow within and between populations (JORDANO et al., 2007), and escaping from density-depend mortality under conspecific trees (CONNELL, 1971; JANZEN, 1970). The escape benefit is an extensively studied process (COMITA et al., 2014) that affects both plant demography and community dynamics. According to the Janzen-Connell hypothesis (CONNELL, 1971; JANZEN, 1970), under parent-tree, seeds are subjected to high density-dependent mortality induced by host-specific pathogens (e.g. fungi and bacteria), seed predators (insects and rodents) and increasing intraspecific competition. Therefore, the dispersal away from parent trees enhances the *per capita* seed survival and establishment probability. It has been shown that the live soil microbiota under conspecific trees, mainly fungi pathogens, are sufficient to induce this high mortality by a process known as the negative plant-soil feedback (NPSF; KLIRONOMOS, 2002; KULMATISKI et al., 2008; MANGAN et al., 2010). The decreasing of conspecific survival and fostering of heterospecific offspring near parent trees has been recognized as an important process that modulates the relative abundance of species (LEVI et al., 2019). Fruit species are thus subjected to the distance- and density-dependent effects. However, while some zoochoric species are negatively affected by the local extinction of frugivores, others thrive relatively well (NUNEZ-ITURRI; OLSSON; HOWE, 2008). This discrepancy in the capacity of plant species to recruit without seed dispersers might be a consequence of the morphological and functional fruit/seed traits that modulate plant species responses to environmental constraints, and consequently their dependence on seed handling by frugivores.

Zoochoric seed dispersal involves the movement of seeds, their treatment in the mouth and/or the gut of frugivores, and their deposition (SCHUPP; JORDANO; GÓMEZ, 2010a; STRINGER et al., 2020). Thus, each frugivore treatment has a differential ecological contribution to the final recruitment probability and survival. First, when frugivores handle the seeds, they clean the seeds from their pulp, removing chemical inhibitors and reducing pathogen attraction (TRAVESET; VERDÚ, 2002). Secondly, the gastrointestinal passage removes the perishable exocarp from intact seeds and cleans the seed testa of the tightly adhered edible fleshy fruit pulp (LEVI; PERES, 2013). It may also contribute to mechanical and chemical scarification that interrupt the seed dormancy (BASKIN; BASKIN, 2014; TRAVESET; RODRÍGUEZ-PÉREZ; PÍAS, 2008; TRAVESET; VERDÚ, 2002). The ingestion (seed cleaning and gastrointestinal passage) by strictly frugivore primates increases the germination success by 75% and accelerates the germination time by 17% (FUZESSY et al., 2016). This fast growth enhances plant survival as the seedlings can capture resources faster and escape early from enemies. Thirdly, ingested seeds deposited in faecal matrixes can enhance their germination through a possible fertilizing effect or decrease it by the presence of microorganisms in faeces (TRAVESET; ROBERTSON; RODRÍGUEZ-PÉREZ, 2007; TRAVESET; VERDÚ, 2002) or by an increased competition when frugivore behaviour results in aggregated seed deposition (RUSSO; AUGSPURGER, 2004). Lastly, germination success and survival can increase with seed removal from the vicinity of parent-trees, where soil microbiota, chemical components and sapling density jeopardize conspecific propagules (CRAWFORD et al., 2019; MANGAN et al., 2010; MCCARTHY-NEUMANN; KOBE, 2010). As a result, seeds can benefit from or be harmed by frugivore handling along the seed dispersal process. Such interaction is a two-way road that depend on the limitations of seeds to recruit and the quality of seed handling by frugivores. By uncovering the causes of variation of plant responses to pulp removal and dispersal away from conspecifics, it would be possible to better predict the effect of the local extinction of dispersers on forest regeneration.

Aslan et al. (2019) proposed to use fruit traits to understand species vulnerabilities to seed mortality and recruitment failures and, therefore, its dependence on dispersal by frugivores. The authors argue that understanding plant characteristic that leads to germination vulnerabilities could help understanding "how much it matters if their seeds are dispersed at all" (ASLAN et al., 2019). Some studies have already associated some seeds characteristics to the plant limitation in germination and survival. Thin seed coat are less resistant to soil enemies (GARDARIN et al., 2010) while thick seed coat may protect the embryo and increase life expectancy of seeds. Moreover, thick seed coat usually result in dormancy, which also imposes germination limitation (BASKIN; BASKIN, 2001) and will require different frugivore treatments such as gut passage or deposition in adequate microsites. However, these studies do not link plant traits with seed dispersal dependence. Doing so, we can better understand the constraints underlying recruitment limitation (TERBORGH et al., 2011). Here, we proposed to estimate the effect of two frugivore treatments, seed cleaning and seed removal away from conspecifics, on the early success of three fruit species with distinct traits, under field conditions. By understanding the differential dependence of seeds on frugivores, we can best predict which treatment is indispensable for the plant early success and, therefore, what to expect in terms of plant responses in the absence of their dispersers.

In this study, we aimed to investigate how seed handling by frugivores affects the recruitment success of three plant species with distinct fruit/seed traits, under field conditions: *Castilla ulei* (Moraceae; fleshy pulp hardly adhered to the soft seed), *Hymenaea parvifolia* (Fabaceae; hard-wood pod, farinaceous pulp and hard seed) and *Byrsonima arthropoda* (Malpighiaceae; fleshy pulp around a hard-wood pyrene with 2-3 seeds) and how much these species depend on such process in early stages. To do so, we i) tested the effect of two frugivore treatments: seed cleaning and removal away from conspecifics, on germination, seedling emergence, and seed/seedling survival; ii) estimated how much each treatment changes the early plant success compared to undispersed seeds. We expected that *C. ulei* would have great increasing in germination and recruitment with seed cleaning as the species has highly adhered fleshy pulp, which attracts seed enemies and thin seed coat, which makes the embryo vulnerable to attacks. We expected that seed cleaning would have positive, but small effect on *B. arthropoda* as the species also has fleshy pulp, but seeds are protected by the pyrene. Seed removal would have secondary or null effect for these species. On the other hand, the seed removal would surpass the importance of seed cleaning for *H. parvifolia* on early success as the species has dry pulp, which is not associated with great attraction of predators and pathogens, and thick seed coat, that protects the embryo. Thus, escaping from the vicinity of conspecific would be sufficient for increasing germination and recruitment probability.

2. METHODS

2.1. STUDY SITE

Field experiment was carried out in continuous lowland rainforest at Fazenda São Nicolau (FSN; 09°52'24"S, 58°13'17"W), Cotriguaçu municipality, Mato Grosso, Brazil (figure 1). The FSN is in the south of Amazon forest, on the left bank of the Juruena River. It extends for a total area of 10,134.43 ha, including 1,781.30 ha of Permanently Protected Area (APP). The climate is tropical, hot, and humid (ANJOS-SILVA, 2010; BRAGA et al., 2017). Mean temperature varies between 23 and 25°C, the average relative humidity reaches 80% and the average annual rainfall is 2300 mm. The typical vegetation is the dense ombrophilous forest with open undergrowth (VELOSO; RANGEL FILHO; LIMA, 1991). Local annual mean precipitation is 2.034 mm (estimated from 2000 to 2009) with the dry season from April to September and raining season from October to March (NORONHA et al., 2015).

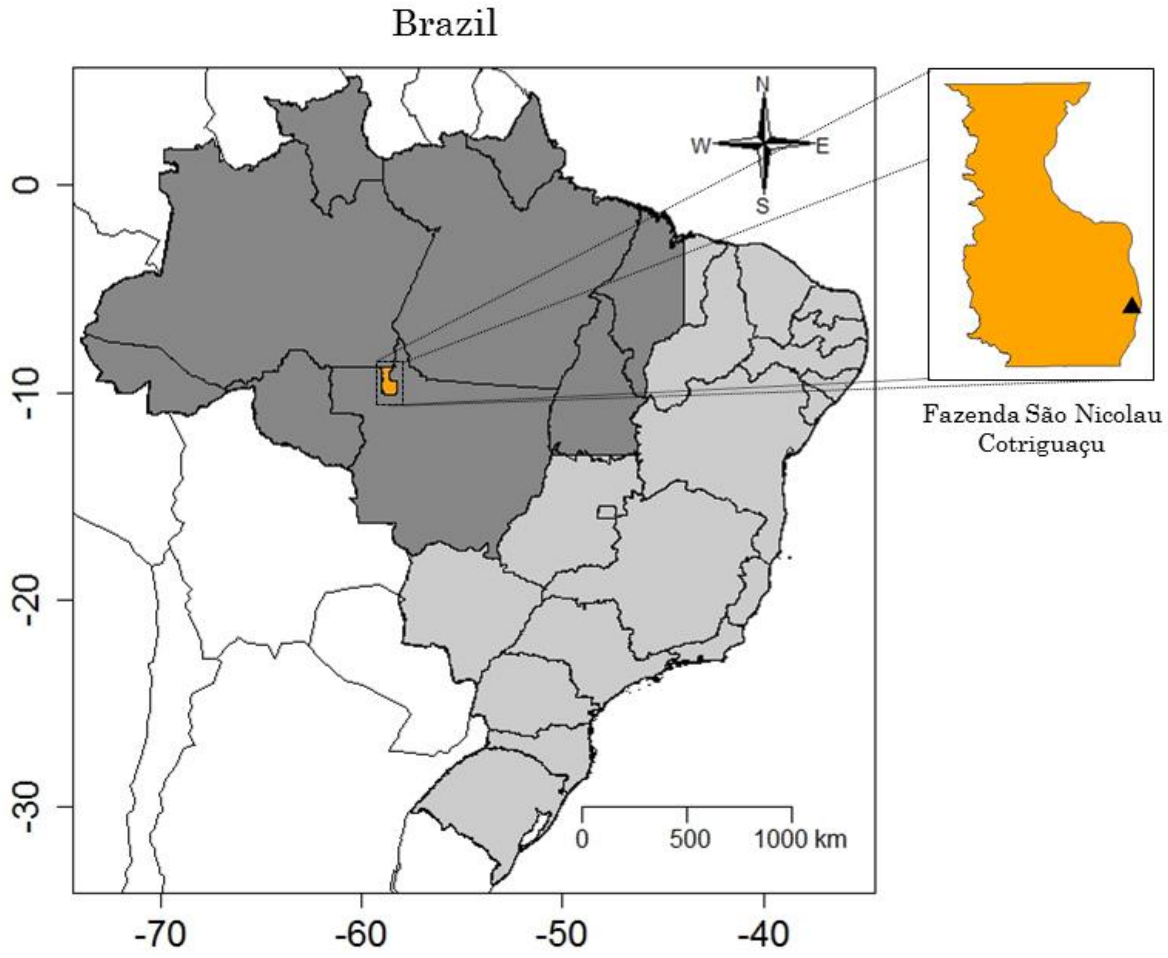

FIGURE 1: Study site located at Fazenda São Nicolau (black trianle), Cotriguaçu municipality (in orange), Mato Grosso, Brazil.

2.2. PLANT SPECIES

We selected three fruit species: *Castilla ulei* (Moraceae), *Hymenaea parvifolia* (Fabaceae) and *Byrsonima arthropoda* (Malpighiaceae). The species had abundant fruit crop during the study period and they are consumed and dispersed by the most sighted (1.8/10km) frugivore at FSN, *Lagothrix cana* (OLIVEIRA et al., 2019).

The species *C. ulei* (popularly known as "caucho") is a tree up to 30 m high (CORNEJO; JANOVEC, 2010). It is a 3-5 cm infructescence, pubescent and yellow to orange when mature (figure 2; CORNEJO; JANOVEC, 2010; YUMOTO; KIMURA; NISHIMURA, 1999). The infructescence has soft pericarp and multiple beige seeds of $\overline{X}$=8.60 mm (±1.90, N=7) in a hardly adhered yellow fleshy pulp that contain 22% of sugar concentration (figure 2; YUMOTO, 1999). It is found from Mexico to

Peru, Bolivia and Brazil. Historically, *C. ulei* had been heavily exploited for rubber latex (CORNEJO; JANOVEC, 2010). The species was recorded being dispersed by Atelinae primates (ARROYO-RODRÍGUEZ et al., 2015; PINTO; SETZ, 2004; STEVENSON et al., 2002) and Salvin's curassow, *Mitu salvini* (Cracidae; YUMOTO; KIMURA; NISHIMURA, 1999). Such species ingest the seeds, cleaning it, and potentially removing it.

H. parvifolia (popularly known as "jatobá-mirim" or "jutaí-mirim") is an emergent tree found in Bolivia, Brazil, Colombia and Peru. Its fruits are 59.6 mm (±1.80, N=20) long, indehiscent heavy woody pods, brown when mature. Each fruit has up to 3 seeds, $\bar{X}$ =25.40 mm (±1.70, N=20; figure 3). Seeds are dark-red, opaque and present a bone consistency (CAMARGO et al., 2008). Seeds are wrapped in a farinaceous pulp. The genus is generally associated to the dependence of dispersal by agoutis (*Dasyprocta spp.*; ASQUITH et al., 1999; GORCHOV; PALMEIRIM; ASCORRA, 2004; HALLWACHS, 1986), which play dual role of predator and disperser by removing the seeds away from parent-tree and bury them.

B. arthropoda is popularly known as "murici" found from Brazil to Nicaragua. Its fruits are round drupe of $\bar{X}$ =12.9 mm long (±1.31, N= 40), yellow when mature (CAMARGO et al., 2008). It has a yellow fleshy pulp and a membranous exocarp. Inside the pericarp, there is a hard-woody pyrene (the diaspora) of $\bar{X}$= 7.3 mm (±0.70, N=40) that protects up to 3 seeds (usually 2; figure 4). Seeds have fragile epicarp with a spiral embryo. The pyrene is the species' propagule. The genus were recorded being dispersed by lizards (Teiidae) that ingest the seeds (MAGNUSSIN; DA SILVA, 1993), and being consumed by the bird *Trogon melanocephalus* (RIEHL; ADELSON, 2008) and the primate *Saimiri sciureus* (STONE, 2007).

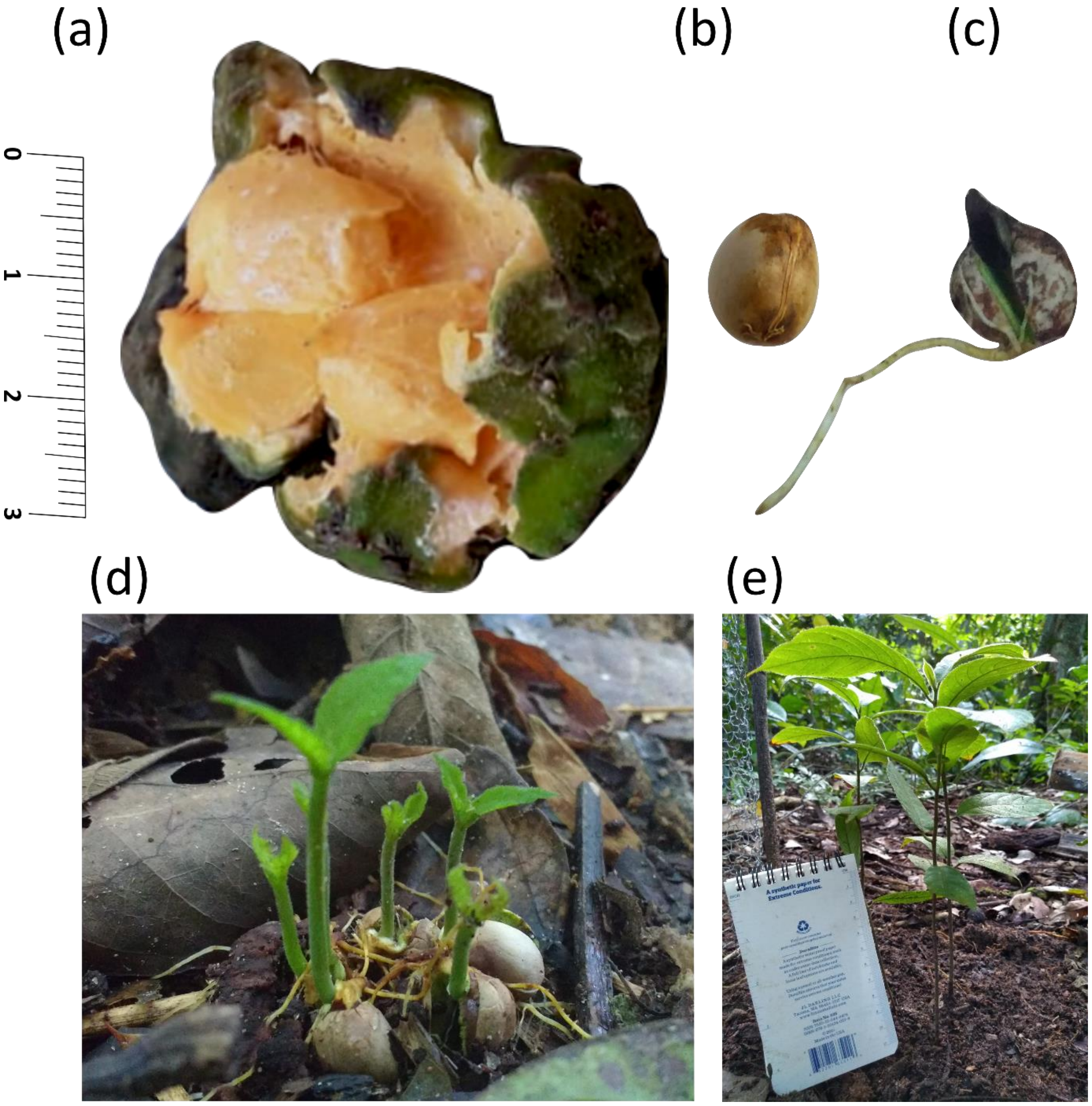

FIGURE 2: Different stages of *Castilla ulei*. (a) Pubescent infructescence with soft mesocarp and multiples seeds stocked in yellow fleshy-pulp, seeds (b) hand-cleaned and (c) germinating, seedlings emergence in (d) 10 days and (e) five months of field experiments.

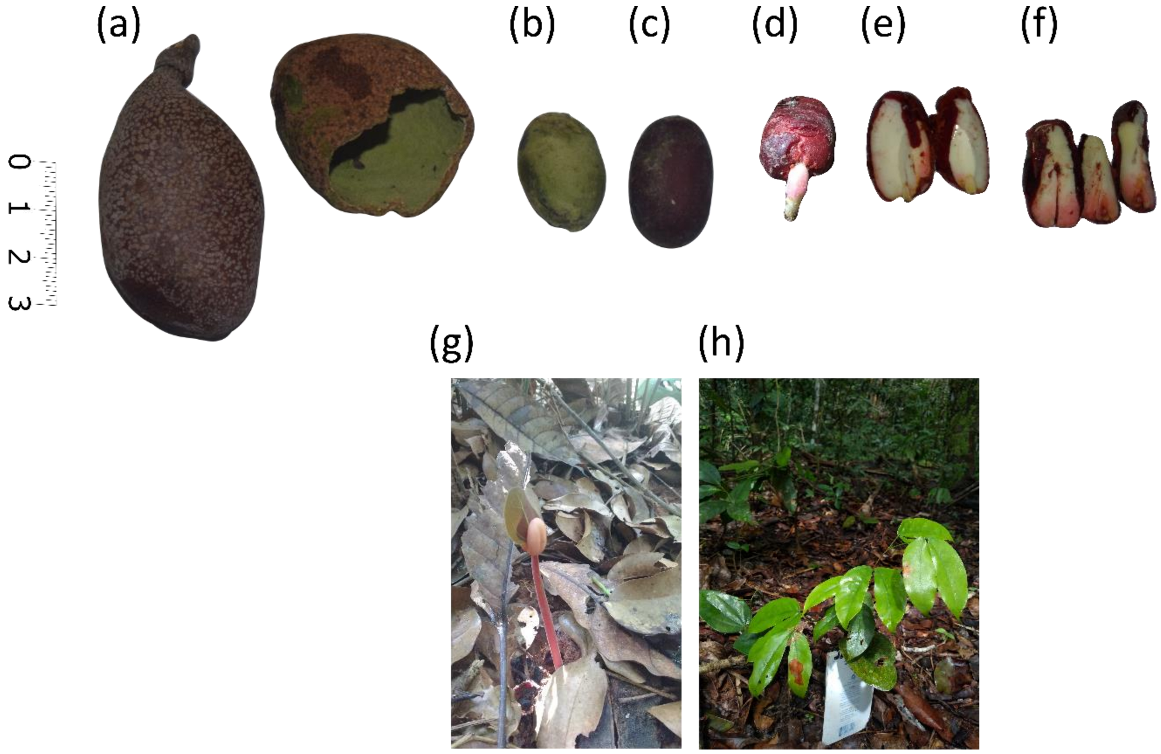

FIGURE 3: Different stages of *Hymenaea parvifolia*. (a) Indehiscent hard-pod with seeds (b) within farinaceous green pulp, (c) hand-cleaned, (d) germinating, with embryos (e) not stained with tetrazolium test – considered dead, (f) stained in red with tetrazolium - considered alive, seedling emergence in (g) one month and (h) in sixteen months of field experiments.

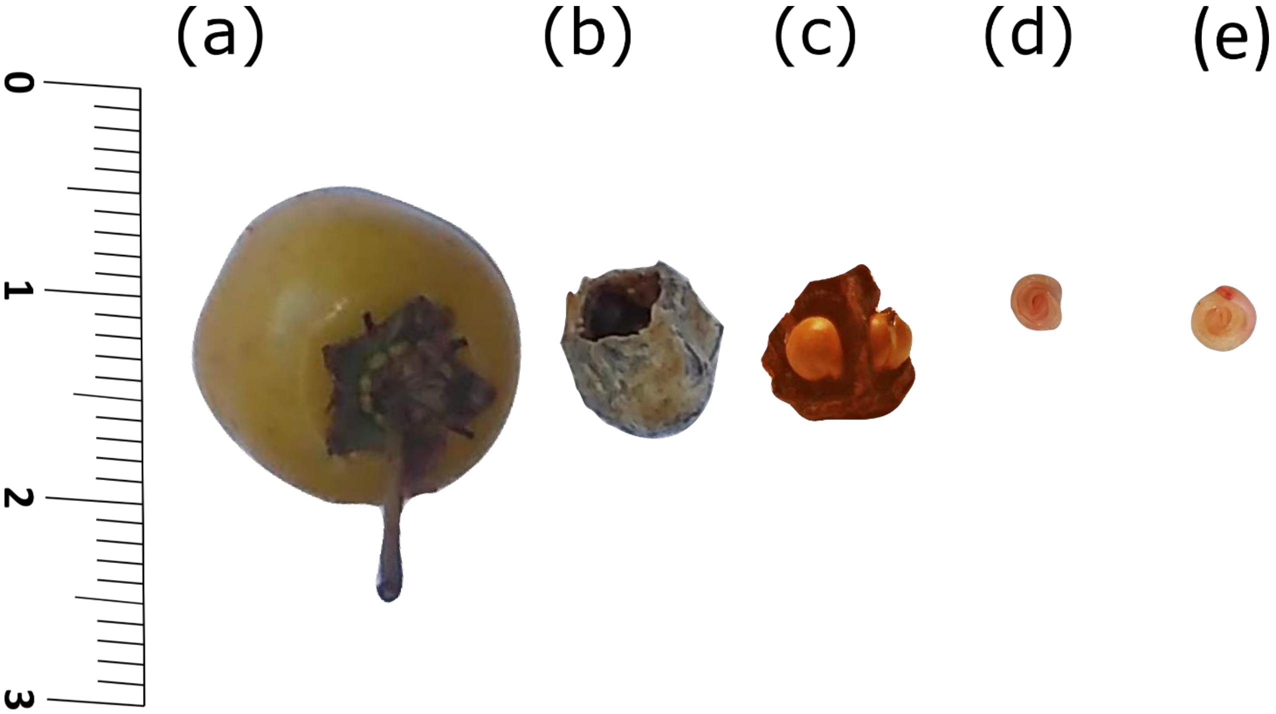

FIGURE 4: Different stages of *Byrsonima arthropoda*. (a) Drupe fruit with yellow fleshy-pulp that have one (b) hard-wood pyrene usually with (c) two seeds within fragile pericarp. (d) Stained embryos in red with tetrazolium solution – considered alive and (e) not-stained embryos – considered dead. We did not record any seed germination or seedling emergence in six months.

2.3. SEED FIELD EXPERIMENTS

We manually collected ripe fruits from the treetop during the fruiting period of each species. We inspected each seed and excluded the ones with visual damages (e.g. bruquids, predation). For *C. ulei* and *H. parvifolia*, we ended up with a total of 800 selected seeds from one individual tree and 800 pyrenes of *B. arthropoda* (1600 seeds) from multiple trees. We hand-cleaned the pulp of half of them, simulating the seed cleaning made by the frugivores.

We set up our experiment in the field using a factorial design (figure 5). We placed 10 seeds of *C. ulei* and *H. parvifolia* and 10 pyrenes of *B. arthropoda* inside a semi-permeable exclosure (to protect seeds against medium and large ground animals) of 20-gauge chicken wire (1 -in. hex mesh), 30 cm-diameter and 50 cm-height, firmly pegged to the ground with bamboo stakes. Each exclosure had one of the following seed treatments: cleaned seeds or seeds with pulp. We then placed four exclosures of each treatment around a conspecific fruiting tree (less than 2 m away) and repeated the same design 20 m away, around a pairwise heterospecific tree. Each pair-wise experimental group (conspecific and heterospecific trees) was placed at least 50 m apart from another pair-wise group. In total, we had 200 seeds of *C. ulei* and *H. parvifolia* and 400 seeds of *B. arthropoda* distributed in 5 pair-wise groups for every possible combination of treatments: seed with pulp or cleaned seeds under conspecific or heterospecific trees. Therefore, we were able to test the effect of seed cleaning (simulating the pulp removal by frugivores) and dispersal away from conspecific trees (simulating the seed removal by frugivores) on three seed life-stages: germination, seedling emergence and survival.

We set up the experiments of *H. parvifolia* at the end of September 2018, *C. ulei* in November 2018 and *B. arthropoda* in early July 2019. We checked the experiments for germination and seedling emergence for up to 331 days in intervals from 10 days to 5 months (table A1 in appendix 1). On each checking date, we recorded germinated seeds, seedling emergence and number of seeds visualized intact. We considered germination when we could see the emission of radicle and seedling emergence when we saw the first leaves. We recovered ungerminated seeds of *H. parvifolia* and *C. ulei* for checking viability in May 2018 (after 8, 7 months respectively). Ungerminated seeds of *B. arthropoda* were checked in February (after 6 months). We checked seed viability through the tetrazolium test (2,3,5 triphenyl tetrazolium chloride 1% solution) in a laboratory. Viable metabolic tissues of seeds soaked in this solution stain pink or red (OOI; AULD; WHELAN, 2004). We, therefore, exposed the seed tissues by cutting the endocarp and then imbibed the seeds in distilled water overnight. They were then placed in the tetrazolium solution in a dark container for 24 hours. Embryos coloured in red were considered viable (figures 3 and 4).

FIGURE 5: Experimental design of the field experiment with two treatments on seeds: seeds with pulp and hand-cleaned seeds and two treatments of deposition sites (removal away): under conspecific trees and under heterospecific trees. We placed 10 seeds in each exclosure for *Castilla ulei* and *Hymenaea parvifolia* and 10 pyrenes (20 seeds) for *Byrsonima arthropoda*. We had four exclosures for each treatment (hand-cleaned seeds and seeds with pulp) under 5 conspecific trees paired with 5 heterospecific trees. In total, we had 200 hand-cleaned seeds under conspecific trees, 200 seeds with pulp under heterospecific trees, 200 hand-cleaned seeds under heterospecific trees and finally, 200 seeds with pulp under conspecific trees for *C. ulei* and *H. parvifolia* and 400 seeds of each treatment combination for *B. arthropoda*.

2.4. DATA ANALYSIS

2.4.1. Seed Field Experiments

We built a life-table (each line corresponding to one seed, the sampling unit) for each stage: germination, seedling emergence and seed/seedling survival of *C. ulei*, *H. parvifolia* and *B. arthropoda* according to Mcnair et al. (2012). Once a seed has germinated, its final status is 1 and the event is the time (in days) of event record. We did the same for seedling emergence and survival (in this case, the status is 1 when seed/seedling dies). To be conservative, we considered seeds that we could not see as dead for the analysis, however, some of these seeds might have been predated or dispersed by small vertebrates. We also run the analyses removing all missing seeds, but the results were similar, therefore, we will report results from analyses considering all seeds.

We compared the success rate between the covariables seed cleaning (treatment cleaned seeds *versus* seeds with pulp) and removal (treatments deposition under conspecific *versus* heterospecific trees) using a semi-parametric model for time-to-event analysis, the Cox proportional hazards model (MCNAIR et al., 2012; THERNEAU; GRAMBSCH, 2000) for *C. ulei* and *H. parvifolia*. The Cox model is based on the hazard function of events (germination, seedling emergence, death) occurring at a time t given the covariables. To test if the hazard functions of the treatments within a covariable are significative different, we used the Efron method as indicated by Mcnair et al. (2012), which estimates the hazard rate (HR) between covariable x_1=1 relative to $x_1 = 0$. We then performed a pairwise comparison with a Chi-square test ($\chi2$) with function "anova.coxph" within significative covariates. We only had data of seed viability of *B. arthropoda* at the end of the experiment, therefore, we ran a generalized linear mixed model (GLMM) with number of viable seeds under a *Poisson* distribution, considering each exclosure (with 20 seeds each) as the sampling unit. For both Cox model and GLMM, we included the variables seed cleaning and removal away as fixed effects and the central tree around which experiments were placed as a random effect. We also included the interaction between the treatments in the model. We chose the best-fitted model by a stepwise model selection, using Akaike information criterion (AIC_c). All analyses were carried out in the statistical software R (v.3.6.1, R Core Team, 2013) using the packages *survival* (THERNEAU, 2015), *nlme* and *AICcmodavg* (MAZEROLLE, 2013).

2.4.2. Change of Seed Dispersal Effectiveness

We measured the quality component of SDE as the probability of seeds and seedlings to survive until the end of the experiment, which occurred after 11 months for *H. parvifolia*, 9 months for *C. ulei* and 6 months for *B. arthropoda* (table A1). To estimate the direction and magnitude of change of reproductive success (SDE *change*) caused by the seed handling by frugivores (seed cleaning and removal away from conspecifics) compared to undispersed seeds, we adapted the method developed by Culot et al. (2015). The SDE *change* is an extension of the SDE landscape proposed by Schupp et al. (2010). The SDE landscape is a graphic representation of the possible combinations of the quantity (x-axis) and quality (y-axis) components of SDE. The value of SDE is obtained by multiplying the values of the x-axis (quantity) by the values of the y-axis (quality), in such a way that a same SDE value is represented by an isocline (see SCHUPP; JORDANO; GÓMEZ, 2010b). The SDE *change* approach enables to access how much the SDE is changed by an increasing probability of a specific event occurs, without the need of the quantity component. Originally, the method was developed to determine the effect of a secondary dispersal event to overall SDE. The change of SDE is obtained by the product of the probability of such event to occur by the proportional change of dispersal quality between the process with and without such event (see details in CULOT; HUYNEN; HEYMANN, 2015 and in the appendix). In the same way as the SDE landscape, a same SDE change (represented as the percentage of increase or decrease of the original value) is represented by an isocline in the graph. Similarly, we used it to access the direction and magnitude of change of plant reproductive success when only seed cleaning ($\mathrm{SDE}_{\mathrm{clean}}$ *change*) or the combination of seed cleaning with removal away from conspecifics ($\mathrm{SDE}_{\mathrm{het}}$ *change*) occur, compared to the reproductive success obtained from undispersed seeds (uncleaned seeds fallen under the parent tree), the null SDE ($\mathrm{SDE}_{\mathrm{null,}}$ figure 6, detailed calculations are in the appendix 2). The SDE change of seed cleaning ($\mathrm{SDE}_{\mathrm{clean}}$ *change*) represents how much the reproductive success of the plant species – if all seeds fall uncleaned under the parent tree ($\mathrm{SDE}_{\mathrm{null}}$) – is increased or decreased by seed cleaning (equation 1).

$$SDE_{clean}change = \frac{SDE_{clean}-SDE_{null}}{SDE_{null}} \text{ x}100 \qquad \text{(equation 1)}$$

Using mathematics simplifications, we have:

$$SDE_{clean}\, change = \frac{Pcc\ (Qucc-Qupc\ )}{Qupc}\text{x}100$$

Where Pcc is the proportion of cleaned seeds deposited under conspecific trees by frugivores (varies from 0 to 1) and is the x-axis of the SDE *change* graph; $Qucc$ is the quality component (probability of seeds/seedlings surviving until the end of the experiment) of the treatment “cleaned seeds under conspecific trees”; $Qupc$ is the quality component of the treatment “seeds with pulp under conspecific trees”. The relation $\frac{(Qucc-Qupc\ )}{Qupc}$ is thus the proportional change of quality caused by the seed cleaning treatment compared to uncleaned seeds and is graphically represented in the y-axis. The multiplication of x-axis and y-axis corresponds to the $SDE_{clean}change$. We then multiplied by 100 to obtain percentage values.

Similarly, the SDE change of removal away from conspecifics ($\mathrm{SDE}_{\mathrm{het}}$ *change*) represents how much the reproductive success of the plant species if all seeds fall uncleaned under the parent tree

(SDE_{null}) is increased or decreased by seed cleaning combined by the removal from conspecifics (equation 2).

$$SDE_{het}change = \frac{SDE_{het} - SDE_{null}}{SDE_{null}} \text{x}100 \qquad \text{(equation 2)}$$

$$SDE_{het}change = \frac{Pch\ (Quch - Qupc)}{Qupc} \text{x}100$$

Where Pch is the proportion of cleaned seeds deposited under heterospecific trees by frugivores (varies from 0 to 1) and is represented in the x-axis. The $Quch$ is the quality component of the treatment "cleaned seeds deposited under heterospecific trees"; $Qupc$ is the quality component of the treatment "seeds with pulp deposited under conspecific trees". The relation $\frac{(Quch - Qupc)}{Qupc}$ is thus the proportional change of quality caused by the seed cleaning combined with the removal away from conspecifics compared to uncleaned seeds deposited under conspecifics and is graphically represented in the y-axis. The multiplication of x-axis and y-axis corresponds to the $SDE_{het}change$. We then multiplied by 100 to obtain percentage values.

Since our objective was to predict the effect of frugivores on seed dispersal effectiveness, we simulated all proportions of seeds handled (Pcc and Pch, from 0 to 1) corresponding to the possible variation observed in different locations and frugivore communities. Doing so, we can visualize how SDE changes with an increasing frugivore activity.

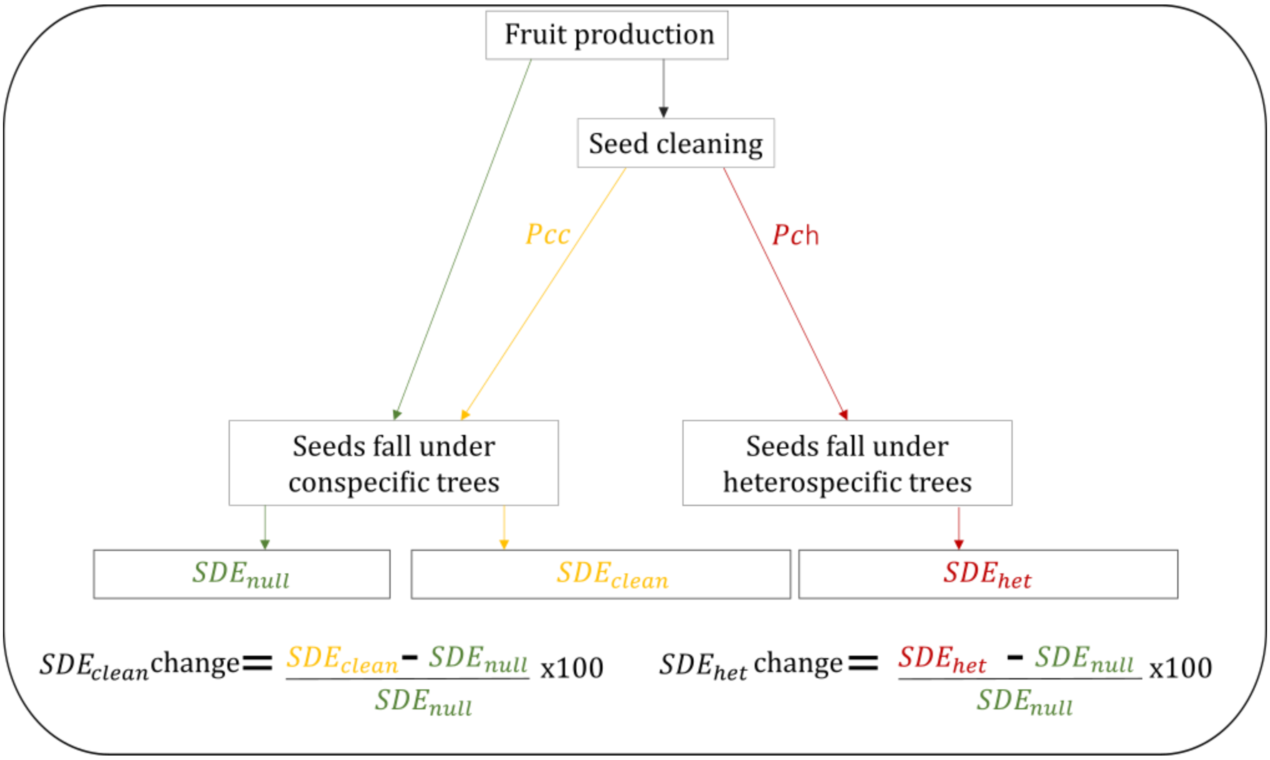

FIGURE 6: SDE$_{null}$ is the expected reproductive success of a plant when there is no seed handling by frugivore, i.e. when all fruit crops fall uncleaned under the parent tree. SDE$_{clean}$ and SDE$_{het}$ are the Seed Dispersal Effectiveness when a proportion of seeds is cleaned and deposited under conspecific trees (Pcc) or cleaned and deposited under heterospecific trees (Pch), respectively. The SDE change of seed cleaning (SDE$_{clean}$ *change*) represents how much the reproductive success of the plant species – if all seeds fall uncleaned under the parent tree (SDE$_{null}$) – is increased or decreased by seed cleaning. The SDE change of removal away from conspecifics (SDE$_{het}$ *change*) represents how much the reproductive success of the plant species – if all seeds fall uncleaned under the parent tree (SDE$_{null}$) – is increased or decreased by seed cleaning combined by the removal from conspecifics.

3. RESULTS

3.1. SEED FIELD EXPERIMENTS

Over the 800 seeds of *C. ulei*, 172 (21.5%) were seen germinated in up to 178 days, when we collected the ungerminated seeds from the field (table 1). We only found 5 empty seed coat and seed fragments at this point. We then considered all ungerminated seeds, 628 (78.5%), as dead in 6 months. They were all rotten or were not seen in the exclosures possibly because they were consumed by small predators or attacked by pathogen. We recorded most germinated seeds

(83.72%) after ten days of experiments. In total, 109 seeds emerged as seedlings up to 297 days (63.4% of seeds that germinated), when we finished monitoring the experiments. None of the seeds with pulp emerged as seedlings. Although 21.5% of the seeds germinated, most of them were from cleaned seeds (table 1), all seeds and most seedlings (99.75%, N=800) died after 10 months. Most seedlings died during the dry season.

After running the stepwise model selection for all combinations of variables for germination, seedling emergence and survival separately, three models had $\Delta AIC_c<2$ (table A2.1) for germination and survival analysis. We then considered the most plausible model as the one with higher weight and smaller AIC_c estimates for each aspect. The best-fitted model for germination included only the seed cleaning treatment, with a strong significant effect (HR= 15.42, CI= 8.91-26.7, *df* =1, χ2=95.48, p<0.001; table A2.2). Cleaned seeds had a higher probability to germinate than seeds with pulp (χ2=184.43, *df*= 1, *p*<0.001; table 1, figure 7). For the seedling emergence, the best-fitted model included all variables: with great significative rate (measure by HR) of seed cleaning (HR=46.08, CI=16.17-131.29, χ2=5143, *p*<0.001) and significative, but low rate of seeds cleaned interaction with removal away (HR=0.2, CI=0.05-0.80, χ2=5.23, *p*<0.001). Cleaned seeds had higher probability to become a seedling than seeds with pulp (χ2=115,83, *df*=1, *p*<0.001; table 1, figure 7). Cleaned seeds under conspecific trees are slightly more likely to emerge as seedlings compared to cleaned seeds under heterospecific trees (χ2=5.45, *df*=0.87, *p*=0.01; table 1, figure 7). The best-fitted model for survival included only the seed cleaning treatment with significant rate (HR=0.6, CI=0.52-0.7, *df*=1, χ2=45.5, *p<0.001*). Cleaned seeds had less probability of dying than seeds with pulp (χ2=46.84, *df*=1, p<0.001; table 1, figure 7).

Over the 800 seeds of *H. parvifolia*, 101 (12.62%) were seen germinated in up to 212 days (table A1). We recorded the first germinated seeds after 21 days of experiment and the first seedlings after 32 days. In 7 months, 308 (38.5%) seeds were lost, mostly due to invertebrates' activity. We recovered 391 intact seeds and tested their viability. Of 391 seeds, 181 (43.86%) were stained with the tetrazolium solution, indicating viable embryos.

Three models had $\Delta AIC_c<2$ (table A2.1) for germination analysis and seedling emergence. All models included for survival analysis were considered plausible. The simplest model that best explained germination probability (AIC_c=1325.3, ΔAIC_c=0, w_t=0.5) included only the removal away from conspecifics (HR=1.54, CI=1.03-2.28, χ2=4.54, *df*=1, *p*=0.03; table A2.2) Seeds deposited under heterospecific trees had higher probability of germinating than under conspecific trees (χ2=4.60, *df*=1, *p*=0.03, table 1; figure 7). The null model was included among the plausible models for seedling emergence (AIC_c=650.5, ΔAIC_c =1.9, w_t=0.2) and survival (AIC_c=6836.8, ΔAIC_c =0.6, w_t=0.2).

We did not record any germination of *B. arthropoda* by 210 days. We collected the 792 (99%) pyrenes – corresponding to 1584 seeds - remaining uninjured in the field for testing the seeds viability. Thus, 8 pyrenes – 16 seeds – were missing and were considered dead. From the recovered ones, 102 (6.4%) seeds were alive. Three models had ΔAIC_c <2 (table A2.1). The simplest model that best explained seed survival included only seed cleaning treatment (ΔAIC_c=0.0, AIC_c=244.6, w_t=0.5). Seeds from cleaned treatment had less probability to survive than seeds from treatment with pulp ($\chi2$= -0.65, SE=0.21, p<0.01; table A2.3, figure 8) under conspecific tree although seed removal was not included in the most plausible model.

TABLE 1: Overall results in absolute number and percentage, in parentheses, of germination, seedling emergence, and survival of *Castilla ulei*, *Hymenaea parvifolia,* and *Byrsonima arthropoda* according to whether the seeds were cleaned or with pulp, and deposited under conspecific or heterospecific trees. We placed a total of 800 seeds in the field of *C. ulei* and *H. parvifolia* (200 for each treatment combination) and 1600 seeds for *B. arthropoda* (400 for each treatment combination).

		GERMINATED SEED			SEEDLINGS EMERGENCE			SURVIVAL		
		Cleaned-seed	Seed with pulp	TOTAL	Cleaned-seed	Seed with pulp	TOTAL	Cleaned-seed	Seed with pulp	TOTAL
C. ulei	***Conspecific***	88 (44)	8 (4)	96 (24)	59 (29.5)	4 (2)	63 (15.7)	3 (1.5)	0 (0)	3 (0.7)
	Heterospecific	69 (34.5)	7 (3)	76 (19)	40 (20)	6 (3)	46 (11.5)	5 (2.5)	0 (0)	5 (1.2)
	TOTAL	157(39.2)	15 (3.7)	172 (21.5)	99 (24.7)	10 (2.5)	109 (13.6)	8 (2)	0 (0)	8 (1)
H. parvifolia	***Conspecific***	19 (9.5)	24 (12)	43 (10.7)	10 (5)	8 (4)	18 (90)	55 (27.5)	76 (38)	131 (32.7)
	Heterospecific	33 (16.5)	25 (12.5)	58 (14.5)	17 (8.5)	14 (7)	31 (15.5)	63 (31.5)	51 (25.5)	114(28.5)
	TOTAL	52 (13)	49 (12.2)	101 (12.6)	27 (6.7)	22 (5.5)	49 (6.1)	118 (29.5)	127 (31.7)	245 (30.6)
B. arthropoda	***Conspecific***	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	14 (3.5)	39 (9.7)	53 (6.6)
	Heterospecific	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	21 (5.2)	28 (7)	49 (6.1)
	TOTAL	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	35 (4.3)	67 (8.3)	102 (6.4)

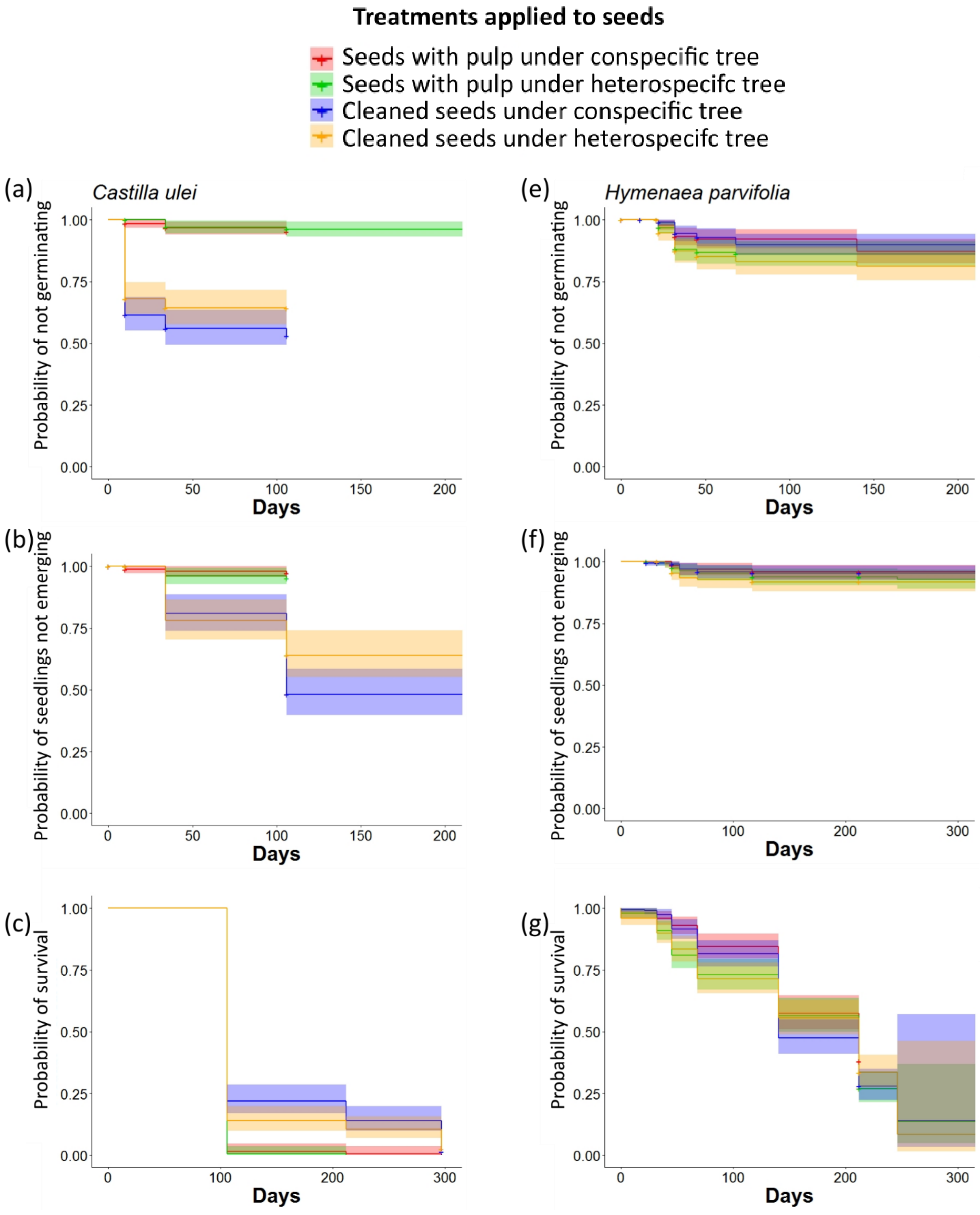
Treatments applied to seeds
Seeds with pulp under conspecific tree
Seeds with pulp under heterospecifc tree
Cleaned seeds under conspecific tree
Cleaned seeds under heterospecifc tree
(a)
Castilla ulei
Probability of not germinating
1.00
0.75
0.50
0.25
0.00
0
50
100
150
200
Days
(b)
Probability of seedlings not emerging
(c)
Probability of survival
0
100
200
300
Days
(e)
Hymenaea parvifolia
Probability of not germinating
(f)
Probability of seedlings not emerging
(g)
Probability of survival

FIGURE 7: Cox proportional hazard model curves of *Castilla ulei* and *Hymenaea parvifolia* showing the probability of not germinating (a,d), seedlings not emerging (b,e) and survival (c,f) through time among the treatments seeds with pulp under conspecific trees (red), seeds with pulp under heterospecifc trees (green), cleaned seeds under conspecifc trees (blue) and cleaned seed under heterospecifc trees (yellow).

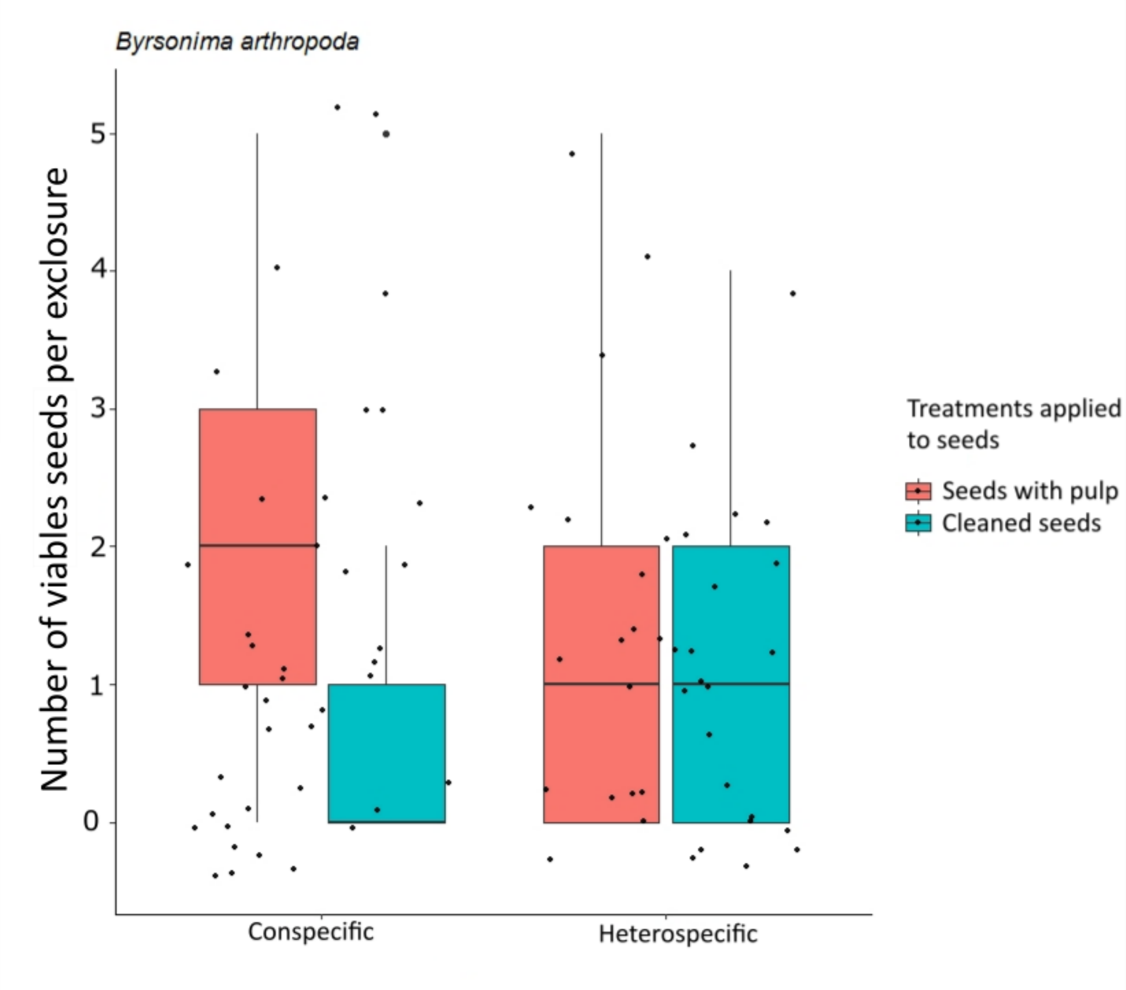

FIGURE 8: Boxplot showing the median of the number of viable seeds (horizontal lines within box) per exclosure (N=20) of *Byrsonima arthropoda*, the interquartile range (boxes), lower and upper limits (vertical lines) and data variation (black points) of treatments “cleaned seeds” and “seeds with pulp” deposited under “conspecifc” and “heterospecific” trees.

3.2. CHANGE OF SEED DISPERSAL EFFECTIVENESS

The direction and magnitude of the effect of seed cleaning and seed cleaning combined with deposition under heterospecific trees relative to SDE_{null} varied among plant species (figure 9). Concerning the effect of seed cleaning by frugivores (SDE_{clean} change) and seed cleaning combined with removal away from conspecifics (SDE_{het} change) on *C. ulei,* the survival of seeds and seedlings of uncleaned seeds under conspecifics were null (Qu_{pc}=0). Therefore, SDE_{clean} change and SDE_{het} change always increase asymptotically its SDE (points outside the graph, figure 9). Despite the positive (but weak) effect of seed removal away from conspecifics on *H. parvifolia* germination, it was not sufficient to positively affect seed/seedling survival, which decreased by up to 25%. Although not significative, seed cleaning treatment also decreased SDE of *H. parvifolia* by >25%. As seed cleaning decreased survival of *B. arthropoda*, the SDE_{clean} reduced SDE by up to 75% as the proportion of seeds handled increases (figure 9), but removal (SDE_{het}), although did not showed significative effect on survival, decreased SDE by only 50%.

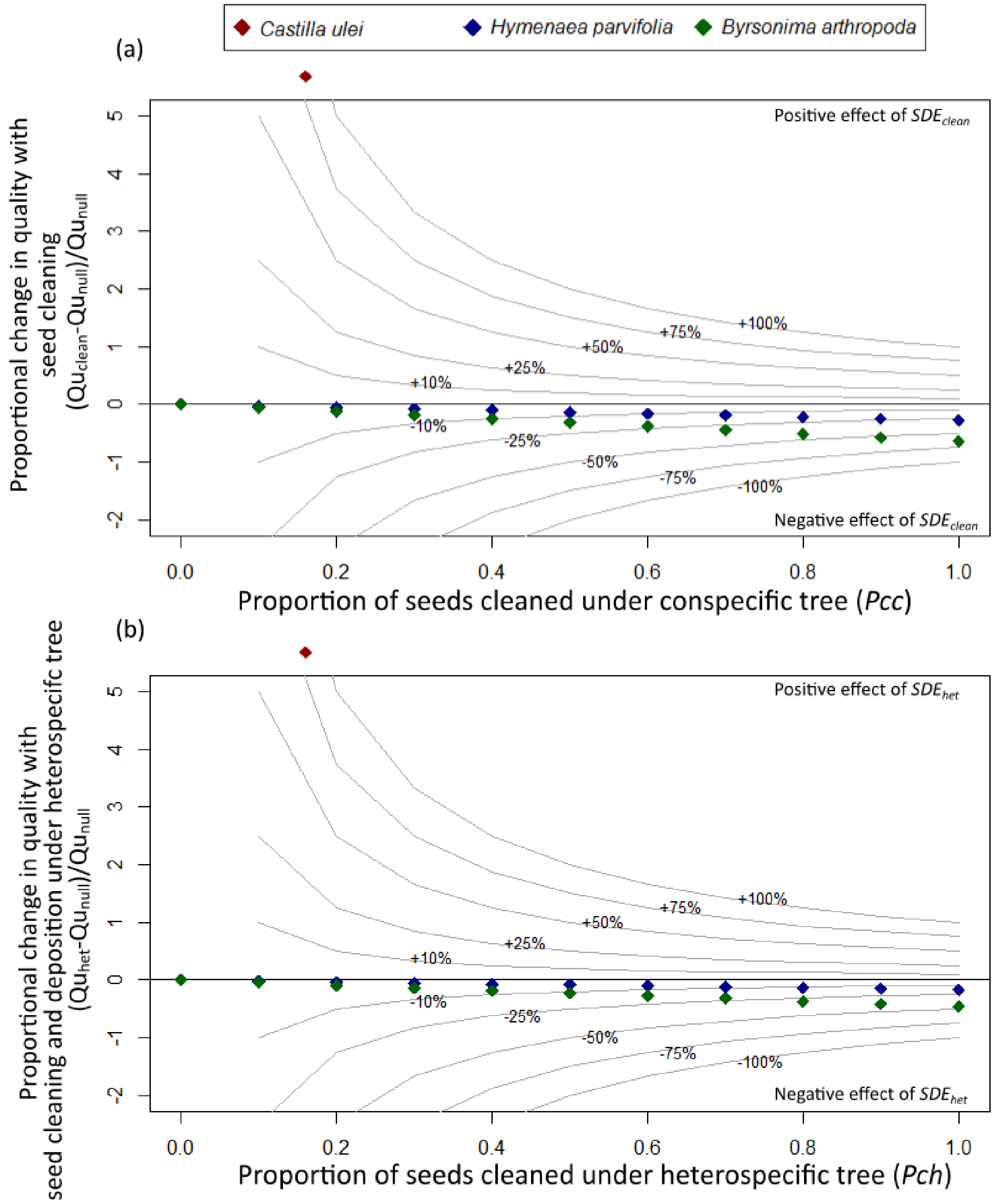

FIGURE 9 : (a) SDE_{clean} change, how much the reproductive success changes with seed cleaning and (b) SDE_{het} change, how much the reproductive success changes with seed cleaning and removal from conspecifics, for *Castilla ulei* (red), *Hymenaea parvifolia* (blue) and *Byrsonima arthropoda* (green). The multiplication between the proportional change of quality (y-axis) and the proportion of seeds handled (x-axis), multiplied by 100, corresponds to the percentage of change of SDE.

4. DISCUSSION

The three plant species we tested responded differently to the seed handling by frugivores. Such discrepancy of plant responses can be explained, at least partly, by the seed traits of species that impose differential constrains to germination, seedling emergence, and survival. Seed cleaning by frugivores have positive effect on the germination and seedling emergence of *C. ulei*, a fleshy fruit with a pulp hardly adhered to the soft seed, but it does not prevent the high rate of seed and seedling death. The deposition of *H. parvifolia* seeds, a fruit with farinaceous pulp and hard seed coat, under heterospecific trees slightly increases germination probability. However, seed cleaning combined with the removal away from conspecifics decreases the reproductive success compared to undispersed seeds. Seed cleaning by frugivores significative decreases the survival probability of *B. arthropoda*, a fleshy fruit with hard-wood pyrene containing soft seeds, indicating that seed handling by frugivores decreases the reproductive success compared to undispersed seeds.

Seed cleaning had great importance on the germination success, seedling emergence and at a lesser extent, survival of *C. ulei*. Deposition under conspecific trees had a secondary effect on the seedling emergence probability. A similar result was found for the common fleshy fruit species *Manilkara bidentata* (LEVI; PERES, 2013) and *Capsicum chacoense* (FRICKE et al., 2013), for which seed cleaning positively affected seedling recruitment. Pulp removal reduces the attraction of pathogens and predators that are accumulated under conspecific trees, especially in the case of fleshy-fruit species. *C. ulei* presents seed traits that make the species more vulnerable in early stages of development. Its soft seed coat is sensitive to desiccation (BASKIN; BASKIN, 2014). Seeds with thin seed coat are more likely to die early, probably because they are less resistant to predation and pathogens and more likely to dry (GARDARIN et al., 2010; NOTMAN; GORCHOV, 2001). The removal of the pulp helps the seeds to escape from enemies attack, thus increasing the species probability of germinate, survive and become seedlings. Fruit traits found in *C. ulei,* such as orange/yellow fruits, indehiscence, juicy pulp and high adherence of pulp were also related to high pathogen attack in an experiment with 16 Peruvian forest species (PRINGLE; ÁLVAREZ-LOAYZA; TERBORGH, 2007). Such characteristics, together with our results, indicate that the species strategy is to have seeds germinating soon after reproduction. Germination period coincides with the beginning of the rainy season in our study site. It requires that seeds have the pulp removed before reaching the ground to enable seeds to germinate fast and not be attacked by predators and pathogens when the soil is more humid.

Despite the great increasing in germination and seedling emergence probability with seed cleaning treatment, we recorded a high mortality of seedlings over the long term. This high mortality suggests that other factors, such as environmental and microsite conditions, highly limit the recruitment of new individuals, a case of life-stage conflict. Even though seedlings can emerge, they may not be able to establish and survive in the long-term due to factors different from the ones affecting germination and seedling emergence. Most seedlings in our study died during the dry season when they were found with yellow and withered leaves. Such deaths can be explained by dehydration or phyto-diseases that attacked and weaken the sprouts or, in some cases, by logs that fell on the exclosures. Experiments under field conditions include multiple uncontrolled variables, especially when there is long temporal variation. Such confounding factors may limit our predictions about the causes of seedling death. However, our results illustrate how the plant early life stages are submitted to different constraints. It is noteworthy that all seedlings that emerged and survived were from cleaned seeds. Such treatment is indispensable for seedling emergence, but it does not prevent the large mortality rate between emergence and establishment. Therefore, large amount of seeds must be cleaned to compensate such seedlings death.

The species *C. ulei* has characteristics associated with primate-dispersed species: yellow/orange colour and high-adherent fleshy-pulp. The hardly adhered pulp influences positively the decision of a frugivore to ingest the seeds to consume the pulp (STEVENSON, 2011b), which helps cleaning it. Moreover, *C. ulei* seeds are medium-sized ($\overline{X}$=8.6 mm ±1.9, N=7), which potentially increases the chances of being ingested by a wider range of bodied-sized frugivores (BUFALO; GALETTI; CULOT, 2016). Our results and inferences highly suggest that the species strongly depend on frugivore to germinate and emerge as seedlings. The genus *Castilla* is generally listed in the diet of large-bodied Ateline primates (ARROYO-RODRÍGUEZ et al., 2015; PINTO; SETZ, 2004; PRUETZ et al., 1998; STEVENSON; QUIÑONES; AHUMADA, 2000; YUMOTO, 1999). In our study site, *Lagothrix cana* and *Ateles chamek* are the most sighted frugivore vertebrates, 1.8/10km and 0.8/10km respectively (OLIVEIRA et al., 2019). We recorded *L. cana* consuming the seeds and we also recovered seeds from their faecal samples. Levi e Peres (2013) estimated that a density of 35.12 ind./km2 of *L. cana* was able to remove

71.27% of fruit production of *M. bidentata*, that has larger fruits (15.5 ± 3.2 mm) and seeds (22.3 ± 4.1 mm) than *C. ulei*. As *C. ulei* produces infructescence with multiple and medium-sized seeds, we predict that *L. cana* at FSN can also remove a high proportion of seeds from *C. ulei*'s tree. However, in a study in Colombia, dispersed seeds (collected from faeces) by *Ateles belzebuth* and *Alouatta seniculus* in the same study significantly reduced germination rate ($p<0.05$). *C. ulei* has thin seed coat, susceptible to soil enemies, thus the faecal matrix may attract a high density of pathogens and seed predators. Thus, Ateline primates have a great potential of cleaning large amount of seeds, increasing the *C. ulei* probability of germination and seedling emergence, however, more studies on gut passage and deposition within faecal matrix would be necessary to test the effect of such treatments on the final germination success of seeds with thin seed coat.

Germination of *H. parvifolia* was slightly increased by the removal away from conspecifics but not by seed cleaning. Seedling emergence and survival were not significantly affected by the treatments. Overall, seed handling by frugivores slightly decreases the reproductive success of the species. *H. parvifolia* seeds are covered by a farinaceous green pulp stocked in an indehiscent heavy and hard pod (CAMARGO et al., 2008). Seeds that fall on the soil covered by pulp are usually cleaned by fungus-cultivating ants Attini (OLIVEIRA et al., 1995), providing germination benefits to the genus *Hymenaea*. We also noticed an intense activity of ants and termites on the soil around the field exclosures. Therefore, we suggest that seeds with pulp were also rapidly cleaned by ants in our study, diminishing the importance of seed cleaning by arboreal frugivores before falling on the ground. Oliveira et al. (1995) observed that uncleaned seeds of *H. courbaril* were mostly infected by fungus, reducing seed viability. In their study, seeds completely cleaned by ants had 68% of germination success in the greenhouse; seeds partially cleaned, 53%; and seeds not cleaned at all had only 18% of germination success. These results combined with ours show that seed cleaning is an important factor to increase seeds´ early success of this species but that it can be accomplished by invertebrates once the seeds fall on the ground.

Seeds of *H. parvifolia* that did not germinate persisted in the seed bank. Seeds from leguminous trees generally have thick seed coat impermeable to water and/or gases (SILVA; CESARINO, 2016). Such characteristics prevents germination due to physical dormancy (BASKIN; BASKIN, 2001), but also prevents seed mortality due to predators and pathogens. Lewinsohn (1980), in a review of seed predation of the genus *Hymenaea*, discusses if the hard seed coat would be an evolutionary response to the high prevalence of invertebrate predators, mainly *Rhinochenus* sp. (Coleoptera: Curculionidae) and Phycitidae (Lepidoptera). These invertebrates are specialized in *Hymenaea* spp. and attack seeds still immature in the tree canopy (LEWINSOHN, 1980). Scolitids (Coleoptera: Scolytidae) and *Microscapus hymenaeae* (Coleoptera: Curculionidae) are also highly prevalent in intact fruits that fall on the soil (LEWINSOHN, 1980). Miller (1991) found that 20% of *H. parvifolia* seeds collected were infested with Curculionids. In our study, we found *Rhinochenus sp.* (Coleoptera: Curculionidae) in 1.05% of seeds (N=1895) and predation marks in 11.45% (N=1895) of screened seeds collected from the treetop. The increase of germination success under heterospecific trees indicate that seeds dispersed away are able to escape from the high prevalence of specific predators and pathogens associated with the genus *Hymenaea*, increasing germination success.

Seed hardness implies a trade-off to seeds. It protects seeds on the soil, however, it prevents germination if dormancy is not broken. The dormancy of *H. parvifolia* is overcome by mechanical or chemical scarification in laboratory conditions (SILVA; CESARINO, 2016). The mechanism whereby physical dormancy is broken in the environment is still unknown (BASKIN; BASKIN, 2001). The dormancy results in slow germination rates and a capacity to persist in seed banks. We indeed found a low germination success for *H. parvifolia* (12%), as so did Miller (1991) in an experiment conducted in a greenhouse (12%) over a longer period (345 days). Interestingly, scarified seeds in their studies had 82-100% of success. We did not investigate the effect of scarification. The passage through frugivore gut contributes to break seed dormancy by reducing water content, thinning the seed coat, or decreasing seed resistance (TRAVESET; RODRÍGUEZ-PÉREZ; PÍAS, 2008). Therefore, despite the none effect of seed cleaning in our study, seeds may have great benefit from ingestion by frugivore to overcome seed dormancy and decrease germination time and seedling establishment. This still needs to be investigated in *H. parvifolia*.

Despite the positive (but weak) effect of seed removal away from conspecifics on *H. parvifolia* germination, it was not sufficient to positively affect seed/seedling survival, which was actually decreased by up to 25%. Frugivore primates are considered the main vertebrate responsible for opening the hard fruits in the treetop and release the seeds (BASKIN; BASKIN, 2001). At FSN, *L. cana* was seen feeding on *H. parvifolia*. The individuals open the fruit, consume the pulp, and spit out most seeds cleaned and uninjured under the parent tree.

However, a few seeds, smaller ($\bar{X}$ =16.6 mm , N=6) than the ones found under trees ($\bar{X}$ =19.5 mm, N=20), were recovered from their faeces samples, indicating that they can occasionally disperse the seeds of this species endozoochorically (pers. obs.). In our study, the seed treatments we tested did not increase *H. parvifolia* SDE, suggesting that this species is not dependent on seed cleaning and removal away does not decrease mortality due to soil enemies. Other groups such as ants (Attini) may clean the seeds once they reach the soil. However, the species still benefit from removal by frugivores to release the seeds from the fruits or to bury it, which also contributes to increase germination probability due to microsite conditions and reduction of pathogens and invertebrates' attacks (ASQUITH et al., 1999) (ASQUITH et al., 1999; GORCHOV; PALMEIRIM; ASCORRA, 2004; HALLWACHS, 1986). As the seed coat is thick, seed scarification through ingestion by large animals such as Ateline primates may also contribute to reduce germination time, which needs further investigation.

We did not record any germination of *B. arthropoda* in seven months. Survival was negatively affected by seed cleaning and removal away from conspecifics did not have any significant effect on seeds. Seed cleaning decreased SDE by up to 75% while seed cleaning combined with removal away decreased SDE by up to 50%. The genus *Byrsonima* has a pyrene diaspora, where small seeds (<5 mm) are stocked in. The woody endocarp acts as a mechanical barrier that prevents seed development (CARVALHO; NASCIMENTO; MÜLLER, 1998). Therefore, we may have missed seeds that eventually germinated in the field and that we were not able to see. Carvalho et al. (1998) also found low germination rate for *B. crassifolia* (11%) tested in a greenhouse. Seeds that did not germinate were still alive. In our study, only 6.4% of seeds were alive. The genus is classified as having physiological dormancy (BASKIN; BASKIN, 2014). Seeds with such dormancy have water-permeable seed coat and require more than four weeks of incubation. Moreover, dormancy is controlled by metabolic and genetic mechanisms. These groups of seeds regulate their germination timing so that it coincides with the most favourable season for seedling establishment (Thompson, 1971 apud BASKIN; BASKIN, 2014). Season changes would be the trigger to germination. The experiment went through the rainy season and we can interpret the negative effect of seed cleaning in two ways. First, seed cleaning exposes the pyrene to water and environmental condition (seeds are water-permeable) in timing not propitious for seed germination. Seeds could have begun to germinate but ended up dying socked in the woody pyrene. Secondly, once cleaned, the pyrenes were more exposed to pathogens which can attack the seeds that present a soft coat, before being able to germinate. Such hypothesis agrees with both cleaning seeds and seeds with pulp having similar survival success under heterospecific trees in our study. We can presume that germination and survival are limited by other factors than dispersal such as local climate and physiological conditions.

B. arthropoda has similar pulp characteristics as *C. ulei*: highly adhered fleshy-pulp, which are generally assigned to the dependence on zoochoric dispersal. Cleaning seeds is sufficient to increase *C. ulei* early success. However, other seed traits such as the presence of pyrenes and dormancy are more important to limit seed survival than pulp removal for *B arthropoda*. Aslan et al. (2019) propose the use of functional groups instead of dispersal syndromes to better understand the vulnerabilities of plant species in the early stages. Dispersal syndromes are more related to the evolutionary and ecological processes underlying the interaction between plant-frugivore. In contrast, functional groups, assigned according to the plants' requirements to recruit, help to better understand the dependence of the plant on different stages of dispersal. Such a framework helps to clarify whether it matters if seeds are dispersed, into what context and with what vectors (ASLAN et al., 2019). According to *B. arthropoda* results we suggest that a group of seed/fruits traits contribute to impose limitation on seed germination and survival that frugivore treatments may not overcome, suggesting that studies should not look only on the pulp characteristics.

Our field experiments showed that the species were affected differently by seed cleaning and seed removal from conspecifics. Such response variation can be interpreted according to the seed characteristics and germination limitation as we discussed above. Comparing the SDE_{clean} (when seeds are cleaned but not removed) and SDE_{het} (when seeds are both cleaned and removed) to SDE_{null} (seeds are not cleaned either removed) help us to understand the actual magnitude and direction of the frugivore treatments on the reproduction success of the studied species.

It is important to highlight that our study tested the effect of distance-based process under population dynamic, such as how escaping from natural enemies concentrated under conspecific trees affects germination, seedling emergence and seed/seedling survival. Another different process, usually mentioned as synonym, is the long-distance dispersal under the community context (SCHUPP; JORDANO; GÓMEZ, 2010a). In this case, the long-distance dispersal contributes to connect metapopulation, colonize new habitat and promote gene flow. Therefore, even when the removal away from conspecific tree was not determinant for initial vital rates in our studies, we cannot disregard the importance of distance-depend dispersal under metapopulation and community processes. Moreover, microsite and environmental factor were not investigated in our study. Such processes highly contribute to the differential probability of seed transition to seedling and adult establishment.

In conclusion, the plant species *C. ulei*, *H. parvifolia* and *B. arthropoda* had different responses to seed cleaning and removal away from conspecifics. Indeed, we also found different responses according to plants life stages. Such responses can be better understood when we investigate the seeds/fruits characteristics. Fruit pulp, seed coat and dormancy type impose different constraints to germination and seed survival. Therefore, according to the species limitations, the seed handling by frugivores affects plant reproduction in different magnitude and in different direction. Moreover, an increased germination success due to seed handling by frugivores has low impact on the final SDE if seeds are not able to survive and recruit as seedlings. Frugivore contribution is the first filter, in long-term, seedling establishment may be more limited by climate conditions or other abiotic factors than process underlying the seed dispersal processes. Understanding such plant limitations and response to different process of frugivore handling help us to better understand the dependence of plant species on seed dispersal. Species such as *C. ulei*, which are highly dependent on large primates for dispersal, are more likely to be negatively affected by defaunation and forest degradation since such vertebrates are preferred targets of hunters and depend on intact forests. In contrast, species like *H. parvifolia*, which dispersal processes can be accomplished by animals like ants or agoutis, which are found even in disturbed habitats, are less likely to be negative affected by the absence of large seed disperser. Our study offers preliminary conclusions about how fruit and seed traits are related to specific responses to handling by frugivores. However, to clearly conclude about general patterns according to plant functional groups, it is necessary to carry out similar studies with other species belonging to the same functional groups and to other ones.

REFERENCES

ANJOS-SILVA, E. J. Eufriesea pulchra Smith (Hymenoptera: Apidae: Euglossini): extended geographic distribution and filling gaps in Mato Grosso state, Brazil. **Neotropical entomology**, v. 39, n. 1, p. 133–136, 2010.

ARROYO-RODRÍGUEZ, V. et al. Seed Dispersal by Howler Monkeys: Current Knowledge, Conservation Implications, and Future Directions. In: **Howler Monkeys: Behavior, Ecology, and Conservation**. [s.l: s.n.]. p. 1–440.

ASLAN, C. et al. Employing plant functional groups to advance seed dispersal ecology and conservation. **AoB PLANTS**, v. 11, n. 2, p. 1–14, 2019.

ASQUITH, N. M. et al. The fruits the agouti ate: Hymenaea courbaril seed fate when its disperser is absent. **Journal of Tropical Ecology**, v. 15, n. 2, p. 229–235, 1999.

BASKIN, C. C.; BASKIN, J. M. **Seeds: Ecology, Biogeography, and, Evolution of Dormancy and Germination**. New Tork: Academic Press, 2001.

BASKIN, C. C.; BASKIN, J. M. **Seeds. Ecology, Biogeography, and Evolution of Dormancy and Germination**. 2nd edn ed. San Diego, CA, USA: Academic Press, 2014.

BRAGA, R. F. et al. Quantification of four different post-dispersal seed deposition patterns after dung beetle activity. **Journal of Tropical Ecology**, v. 33, n. 6, 2017.

BUFALO, F. S.; GALETTI, M.; CULOT, L. Seed Dispersal by Primates and Implications for the Conservation of a Biodiversity Hotspot, the Atlantic Forest of South America. **International Journal of Primatology**, v. 37, n. 3, p. 333–349, 2016.

CAMARGO, J. L. C. et al. **Guia de Propágulos e Plântulas da Amazônia**. Manaus: [s.n.]. v. 1

CARVALHO, J. E. U. DE; NASCIMENTO, W. M. O. DO; MÜLLER, C. H. Características físicas e de Germinação de Sementes de Espécies frutíferas Nativas da Amazônia. **Embrapa Boletim de Pesquisa**, n. 203, p. 18, 1998.

CHAMBERS, J. C.; MACMAHON, J. A. A day in the life of a seed: Movements and fates of seeds and their implications for natural and managed systems. **Annual Review of Ecology and Systematics**, v. 25, p. 263–292, 1994.

COMITA, L. S. et al. Testing predictions of the Janzen-Connell hypothesis: A meta-analysis of experimental evidence for distance- and density-dependent seed and seedling survival. **Journal of Ecology**, v. 102, n. 4, p. 845–856, 2014.

CONNELL, J. H. On the role of natural enemies in preventing competitive exclusion in some marine animals and in rain for- est trees. In: DEN BOER, P. J.; GRADWELL, G. R. (Eds.). . **Dynamics of populations**. Wageningen ed. Wageningen, The Netherlands: Centre for Agricultural Publishing and Documentation, 1971. p. 298–313.

CORNEJO, F.; JANOVEC, J. **Seeds of Amazonian plants**. United States of America. Princeton University Press., 2010.

CRAWFORD, K. M. et al. When and where plant-soil feedback may promote plant coexistence : a meta-analysis. **Ecology Letters**, 2019.

CULOT, L.; HUYNEN, M. C.; HEYMANN, E. W. Partitioning the relative contribution of one-phase and two-phase seed dispersal when evaluating seed dispersal effectiveness. **Methods in Ecology and Evolution**, v. 6, n. 2, p. 178–186, 2015.

FRICKE, E. C. et al. When condition trumps location: Seed consumption by fruit-eating birds removes pathogens and predator attractants. **Ecology Letters**, v. 16, n. 8, p. 1031–1036, 2013.

FUZESSY, L. F. et al. How do primates affect seed germination? A meta-analysis of gut passage effects on neotropical plants. **Oikos**, v. 125, n. 8, p. 1069–1080, 2016.

GARDARIN, A. et al. Seed mortality in the soil is related to seed coat thickness. **Seed Science Research**, v. 20, n. 4, p. 243–256, 2010.

GORCHOV, D. L.; PALMEIRIM, J. M.; ASCORRA, C. F. Dispersal of seeds of Hymenaea courbaril (Fabaceae) in a logged rain forest in the Peruvian Amazonian. **Acta Amazonica**, v. 34, n. 2, p. 251–259, 2004.

HALLWACHS, W. Agoutis (Dasyprocta punctata): The Inheritors of Guapinol (Hymenaea courbaril: Leguminosae). p. 285–304, 1986.

HOWE, H. F.; SMALLWOOD, J. Ecology of Seed Dispersal. **Annual Review of Ecology and Systematics**, v. 13, p. 201–228, 1982.

IBÁNEZ, I. et al. Predicting Biodiversity Change : Outside the Climate. **Ecology**, v. 87, n. 8, p. 1896–1906, 2006.

JANZEN, D. H. Herbivores and the number of tree species in tropical forests. **The American Naturalist**, v. 104, n. 940, p. 501–528, 1970.

JORDANO, P. Fruits and frugivory. In: FENNER, M. (Ed.). . **Seeds: the ecology of regeneration in plant communities**. 2nd. ed. Wallingford, UK: CABI Publishing, 2000. p. 125–166.

JORDANO, P. et al. Differential contribution of frugivores to complex seed dispersal patterns. **Proceedings of the National Academy of Sciences of the United States of America**, v. 104, n. 9, p. 3278–3282, 2007.

KLIRONOMOS, J. N. Feedback with soil biota contributes to plants rarity and and invasiveness in communities. **Nature**, v. 417, n. May, p. 67–69, 2002.

KULMATISKI, A. et al. Plant-soil feedbacks: A meta-analytical review. **Ecology Letters**, v. 11, n. 9, p. 980–992, 2008.

LEVI, T. et al. Tropical forests can maintain hyperdiversity because of enemies. **Proceedings of the National Academy of Sciences**, v. 116, n. 2, p. 581–586, 2019.

LEVI, T.; PERES, C. A. Dispersal vacuum in the seedling recruitment of a primate-dispersed Amazonian tree. **Biological Conservation**, v. 163, p. 99–106, 2013.

LEWINSOHN, T. M. **Predação de Sementes em Hymenaea (LEGUMINOSAE: CAESALPINIOIDEAE): Aspectos Ecológicos e Evolutivos**. Dissertação (Mestrado em Biologia) - Instituto de Biologia, Universidade Estadual de Campinas. São Paulo. p. 202. 1980.

MAGNUSSIN, W. E.; DA SILVA, E. V. Relative Effects of Size , Season and Species on the Diets of Some Amazonian Savanna Lizards. **Journal of herpetology**, v. 27, n. 4, p. 380–385, 1993.

MANGAN, S. A. et al. Negative plant-soil feedback predicts tree-species relative abundance in a tropical forest. **Nature**, v. 466, n. 7307, p. 752–755, 2010.

MAZEROLLE, M. J. Model selection and multimodel inference based on (Q) AIC (c). R package, version 1.35. 2013.

MCCARTHY-NEUMANN, S.; KOBE, R. K. Conspecific plant-soil feedbacks reduce survivorship and growth of tropical tree seedlings. **Journal of Ecology**, v. 98, n. 2, p. 396–407, 2010.

MCNAIR, J. N. et al. How to analyse seed germination data using statistical time-to-event analysis: non-parametric and semi-parametric methods. **Seed Science Research**, v. 22, n. 2, p. 77–95, 2012.

MILLER, R. P. **Dinâmica da Regeneração Natural de Jutaí Mirim (Hymenaea parvifolia Huber - Leguminosae Caesalpinioideae) na Ilha de Maracá- Roraima**. Dissertação (Mestrado em Manejo Florestal) - Pós-Graduação em Biologia Tropical e Recursos Naturais, INPA. Manaus. p. 95. 1991.

NORONHA, J. C. et al. Update das espécies de anuros da Fazenda São Nicolau , Mato Grosso , Brasil. **Scientific Electronic Archives**, v. 8, n. Id, p. 15–25, 2015.

NOTMAN, E.; GORCHOV, D. L. Lowland tropical forest and fallow agricultural sites. **Biotropica**, v. 33, n. 4, p. 621–636, 2001.

NUNEZ-ITURRI, G.; OLSSON, O.; HOWE, H. F. Hunting reduces recruitment of primate-dispersed trees in Amazonian Peru. **Biological Conservation**, v. 141, n. 6, p. 1536–1546, 2008.

OLIVEIRA, A. T. M. et al. Primate and ungulate responses to teak agroforestry in a southern Amazonian landscape. **Mammalian Biology**, v. 96, p. 45–52, 2019.

OLIVEIRA, P. S. et al. Seed cleaning by Mycocepurus goeldii ants (Attini) facilitates germination in Hymenaea coubaril (Caesalpinaceae). **Biotropica**, v. 27, n. 4, p. 518–522, 1995.

OOI, M.; AULD, T.; WHELAN, R. Comparison of the cut and tetrazolium tests for assessing seed viability: a study using Australian native Leucopogon species. **Ecological Management and restoration**, v. 5, n. 2, p. 130–153, 2004.

PINTO, L. P.; SETZ, E. Z. F. Diet of Alouatta belzebul discolor in an amazonian rain forest of Northern Mato Grosso State, Brazil. **International Journal of Primatology**, v. 25, n. 6, p. 1197–1211, 2004.

PRINGLE, E. G.; ÁLVAREZ-LOAYZA, P.; TERBORGH, J. Seed characteristics and susceptibility to pathogen attack in tree seeds of the Peruvian Amazon. **Plant Ecology**, v. 193, p. 211–222, 2007.

PRUETZ, J. D. et al. Seed dispersal of a pioneer tree species (Castilla elastica) by howler monkeys (Alouatta palliata) in Costa Rica. **American Journal of Primatology**, v. 45, n. 2, p. 201, 1998.

RIEHL, C.; ADELSON, G. S. Seasonal insectivory by black-headed trogons, a tropical dry forest frugivore. **Journal of Field Ornithology**, v. 79, n. 4, p. 371–380, 2008.

RUSSO, S. E.; AUGSPURGER, C. K. Aggregated seed dispersal by spider monkeys limits recruitment to clumped patterns in Virola calophylla. **Ecology Letters**, v. 7, n. 11, p. 1058–1067, 2004.

SCHUPP, E. W. Quantity, quality and the effectiveness of seed dispersal by animals. **Vegetatio**, v. 107, n. 1, p. 15–29, 1993.

SCHUPP, E. W.; JORDANO, P.; GÓMEZ, J. M. Seed dispersal effectiveness revisited: A conceptual review. **New Phytologist**, v. 188, n. 2, p. 333–353, 2010a.

SCHUPP, E. W.; JORDANO, P.; GÓMEZ, J. M. Seed dispersal effectiveness a conceptual review. **New Phytologist**, v. 188, n. 2, p. 333–353, 2010b.

SILVA, B. M. S.; CESARINO, F. Germinação de sementes e emergência de plântulas de jutaí (Hymenaea parvifolia Huber.). **Revista Brasileira de Plantas Medicinais**, v. 18, n. 1, p. 256–263, 2016.

STEVENSON, P. R. et al. Effects of seed dispersal by three ateline monkey species on seed germination at Tinigua National Park, Colombia. **International Journal of Primatology**, v. 23, n. 6, p. 1187–1204, 2002.

STEVENSON, P. R. The abundance of large ateline monkeys is positively associated with the diversity of plants regenerating in neotropical forests. **Biotropica**, v. 43, n. 4, p. 512–519, 2011a.

STEVENSON, P. R. Pulp – seed attachment is a dominant variable explaining legitimate seed dispersal: a case study on woolly monkeys. **Oecologia**, v. 12, n. 18, p. 693–701, 2011b.

STEVENSON, P. R.; QUIÑONES, M. J.; AHUMADA, J. A. Influence of fruit availability on ecological overlap among four neotropical primates at Tinigua National Park, Colombia. **Biotropica**, v. 32, n. 3, p. 533–544, 2000.

STONE, A. I. Responses of Squirrel Monkeys to Seasonal Changes in Food Availability in an Eastern Amazonian Forest. **American Journal of Primatology 69:142–157**, v. 69, p. 142–157, 2007.

STRINGER, S. D. et al. Assessing the role of a mammalian frugivorous species on seed germination potential depends on study design: A case study using wild samango monkeys. **Acta Oecologica**, v. 106, p. 103584, 2020.

TERBORGH, J. et al. Decomposing dispersal limitation: Limits on fecundity or seed distribution? **Journal of Ecology**, v. 99, n. 4, p. 935–944, 2011.

THERNEAU, T. **A Package for Survival Analysis in S_. version 2.38**, 2015. Disponível em: <https://cran.r-project.org/package=survival>

THERNEAU, T. M.; GRAMBSCH, P. M. **Modeling Survival Data: Extending the Cox Model**. 1st. ed. New York, NY: Springer-Verlag, 2000. v. 97

TRAVESET, A.; ROBERTSON, A. W.; RODRÍGUEZ-PÉREZ, J. A review on the role of endozoochory in seed germination. In: **Seed Dispersal: Theory and its Application in a Changing World**. [s.l.] CABI, 2007. p. 78–103.

TRAVESET, A.; RODRÍGUEZ-PÉREZ, J.; PÍAS, B. Seed trait changes in dispersers' guts and consequences for germination and seedling growth. **Ecology**, v. 89, n. 1, p. 95–106, 2008.

TRAVESET, A.; VERDÚ, M. A meta-analysis of the Effect of Gut Treatment on Seed Germination. In: **Seed dispersal and frugivory: Ecology, evolution and conservation**. Wallingford, UK: CABI Publishing, p. 339–350, 2002.

VELOSO, H. P.; RANGEL FILHO, A. L. R.; LIMA, J. C. A. **Classificação da Vegetação Brasileira Adaptada a um Sistema Universal**. IBGE, Rio de Janeiro, p. 124, 1991.

YUMOTO, T. Seed Dispersal by Salvin's Curassow,Mitu salvini (Cracidae) in a Tropical Forest of Colombia: Direct Measurements of Direct Dispersal Distance. **Biotropica**, v. 31, n. 4, p. 654–660, 1999.

YUMOTO, T.; KIMURA, K.; NISHIMURA, A. Estimation of the retention times and distances of seed dispersed by two monkey species, Alouatta seniculus and Lagothrix lagotricha, in a Colombian forest. **Ecological Research**, v. 14, n. 2, p. 179–191, 1999.

APPENDIX 1: SUPPLEMENTARY DESCRIPTION OF FIELD EXPERIMENT MONITORING PERIOD

TABLE A1: Experiments checking dates of *Castilla ulei*, *Hymenaea parvifolia* and *Byrsonima arthropoda*. First date is the experiments set up and in bold, the moment we tested the viability of ungerminated seed.

			2018				2019		2020			
H. parvifolia	30/Sept	11/Oct	21/Oct	01/Nov	14/Nov	07/Dec	**30/Apr**	03/June	-	-	27/Aug	-
C. ulei	-	-	-	03/Nov	13/Nov	07/Dec	**30/Apr**	03/June	-	-	27/Aug	-
B. arthropoda	-	-	-	-	-	-	-	-	03/July	13/July	25/Aug	**28/Jan**

APPENDIX 2: DETAILED CALCULATION OF THE CHANGE OF SEED DISPERSAL EFFECTIVENESS

To estimate the direction and magnitude of change of reproductive success (SDE *change*) caused by the seed handling by frugivores (seed cleaning and removal away from conspecifics) compared to undispersed seeds, we adapted the method developed by Culot et al. (2015).

The SDE change of seed cleaning (SDE_{clean} *change*) estimates how much the reproductive success of the plant species – if all seeds fall uncleaned under the parent tree (SDE_{null}) – is increased or decreased by seed cleaning. It represents frugivores that consume the pulp (thus cleaning the seeds) but discard them under conspecific. The SDE change of seed cleaning combined with seed removal (SDE_{het} *change*) estimates how much the reproductive success of the plant species – if all seeds fall uncleaned under the parent tree (SDE_{null}) – is increased or decreased by seed cleaning and deposition under heterospecific tree. It represents frugivores that consume the pulp (thus cleaning the seeds) and deposit then under heterospecific. SDE_{clean} is estimated as the quantity (Qt, fruit crop size) multiplied by the quality component (probability of seeds/seedlings surviving until the end of the experiment) of the treatment “cleaned seeds under conspecific trees” ($Qucc$) and the quality of treatment “seeds with pulp under conspecific trees” ($Qupc$) weighted by the proportion of cleaned seeds, thus handled, that are deposited under conspecifics trees (Pcc) and the proportion of seeds not handled, that falls with pulp under conspecific tree (Ppc), respectively. The sum of Pcc and Ppc is equal to 1. Similarly, SDE_{het} is estimated as the quantity (Qt) multiplied by the quality of the treatment “cleaned seeds under heterospecific trees” ($Quch$) and the quality of treatment “seeds with pulp under conspecific trees” ($Qupc$) weighted by the proportion of cleaned seeds deposited under heterospecific trees (Pch), thus consumed and removed away and, the proportion of seeds with pulp that falls under conspecific tree (Ppc), thus not handled, respectively. The sum of Pch and Ppc is equal to 1.

Finally, we estimated the percentual changes of SDE_{clean} and SDE_{het} relative to the null SDE (SDE_{null}), the plant reproductive success obtained in the absence of frugivore handling, thus all seeds are undispersed (uncleaned seeds fallen under the conspecific tree, Ppc=1). The SDE_{null} is estimated as the product of the quantity (Qt) multiplied by the quality of the treatment “seeds with pulp under conspecific trees” ($Qupc$).

Doing so we can compare the magnitude and direction of treatments changes in SDE of plant reproductive success in the absence of frugivore. The SDE *change* approach enables remove the quantity component (Qt) of original SDE with mathematics simplification.

Calculations were done as follow:

$$SDE = Qt * Qu$$

SDE is estimated as the quantity component multiplied by the quality component. Similarly,

$$SDE_{clean} = Qt\ [Ppc * Qupc + Pcc * Qucc]$$

In SDE_{clean} the seeds handled are cleaned but do not benefit from deposition under heterospecific trees, Ppc + Pcc = 1.

$$SDE_{het} = Qt\ [Ppc * Qupc + Pch * Quch]$$

In SDE_{het} the seeds handled are cleaned and deposited under heterospecific by frugivore. Thus, Pcc = 0 and Ppc + Pch = 1.

$$SDE_{null} = Qt[Ppc * Qupc]$$

In SDE_{null}, no seeds are handled. As Ppc = 1, all seeds fall uncleaned under conspecific tree, we have:

$$SDE_{null} = Qt * Qupc$$

The $SDE_{clean}change$ was calculated as the percentage change of SDE_{clean} relative to SDE_{null} as follow:

$$SDE_{clean}change = \frac{SDE_{clean} - SDE_{null}}{SDE_{null}}\ x\ 100$$

$$SDE_{clean}change = \frac{Qt\ [Ppc * Qupc + Pcc * Qucc] - Qt * Qupc}{Qt * Qupc}\ x\ 100$$

Using mathematic simplification, we can remove *Qt* as follow:

$$SDE_{clean}change = \frac{Ppc * Qupc + Pcc * Qucc - \ Qupc}{Qupc}\ x\ 100$$

As $Ppc = \ 1 - Pcc$, we have:

$$SDE_{clean}change = \frac{(1 - Pcc) * Qupc + Pcc * Qucc - \ Qupc}{Qupc}\ x\ 100$$

$$SDE_{clean}change = \frac{Qupc - Qupc * Pcc + Pcc * Qucc - Qupc}{Qupc}\ x\ 100$$

$$SDE_{clean}change = \frac{-Qupc * Pcc + Pcc * Qucc}{Qupc}\ x\ 100$$

$$SDE_{clean}change = \frac{Pcc\ (Qucc - Qupc)}{Qupc}\ x\ 100$$

The $SDE_{het}change$ was calculated as the percentage change of SDE_{het} relative to SDE_{null} as follow:

$$SDE_{het}change = \frac{SDE_{het} - SDE_{null}}{SDE_{null}}$$

$$SDE_{het}change = \frac{Qt[Ppc * Qupc + Pch * Quch] - Qt * Qupc}{Qt * Qupc}$$

We can remove *Qt*:

$$SDE_{het}change = \frac{Ppc * Qpc + Pch * Quch - \ Qupc}{Qupc}$$

As $Ppc = \ 1 - Pch$, we have:

$$SDE_{het}change = \frac{(1 - Pch) * Qupc + Pch * Quch - \ Qupc}{Qupc}$$

$$SDE_{het}change = \frac{Qupc - Qupc * Pch + Pch * Quch - Qupc}{Qupc}$$

$$SDE_{het}change = \frac{-Qupc * Pch + Pch * Quch}{Qupc}$$

$$SDE_{het}change = \frac{Pch\ (Quch - Qupc)}{Qupc}$$

APPENDIX 3: SUPPLEMENTARY TABLES

TABLE A2.1: Models ranking according to the *Akaike information criterion* (AIC_c) for germination, seedling emergence and survival for the Cox proportional hazards models of *Castilla ulei* and *Hymenaea parvifolia* and the generalized linear mixed models (GLMM) of *Byrsonima arthroproda*. We included the variables seed cleaning (cleaned seeds and seeds with pulp) and removal from conspecifics (under conspecific tree and under heterospecific tree) as fixed effect and the central tree as the random effect. We considered the models with ΔAIC_c <2 as the most plausible. When we had more the one model with ΔAIC_c <2, we chose the one with fewer variables.

		Models	K	AIC_c	ΔAIC_c	ω_t	Cum.W_t
C.ulei	GERMINATION						
		Cleaning + deposition	**7**	**2042**	**0**	**0.5**	**0.5**
		Cleaning * deposition	**8**	**2043**	**1**	**0.3**	**0.7**
		Cleaning	**6**	**2043.1**	**1.1**	**0.3**	**1**
		Deposition	6	2228.2	186.2	0	1
		Null	5	2229	187	0	1
	SEEDLINGS						
		Cleaning *deposition	**8**	**1074.3**	**0**	**0.8**	**0.8**
		Cleaning + deposition	7	1078	3.7	0.1	1
		Cleaning	6	1081	6.7	0	1
		Null	5	1226.1	151.9	0	1
		Deposition	6	1227.6	153.3	0	1
	SURVIVAL						
		Cleaning	**6**	**9041**	**0**	**0.4**	**0.4**
		Cleaning + deposition	**7**	**9041.3**	**0.3**	**0.4**	**0.8**
		Cleaning*deposition	**8**	**9042.7**	**1.7**	**0.2**	**1**
		Null	5	9083.2	42.2	0	1
		Deposition	6	9083.2	42.3	0	1
H. parvifolia	GERMINATION						
		Deposition	**6**	**1325.3**	**0**	**0.5**	**0.5**
		Cleaning*deposition	**8**	**1327**	**1.8**	**0.2**	**0.6**
		Cleaning + deposition	**7**	**1327.2**	**1.9**	**0.2**	**0.8**
		Null	5	1327.8	2.6	0.1	1
		Clean	6	1329.8	4.5	0	1
	SEEDLINGS						
		Deposition	**6**	**648.7**	**0**	**0.5**	**0.5**
		Cleaning + deposition	**7**	**1327.2**	**1.6**	**0.2**	**0.7**
		Null	**5**	**650.3**	**1.9**	**0.2**	**0.8**
		Cleaning	6	652.2	3.5	0.1	0.9
		Cleaning*deposition	8	1327	3.7	0.1	1
	SURVIVAL						
		Cleaning*deposition	**8**	**6836.2**	**0**	**0.3**	**0.3**
		Null	**5**	**6836.8**	**0.6**	**0.2**	**0.5**
		Deposition	**6**	**6837.3**	**0.8**	**0.2**	**0.7**

		Cleaning	**6**	**6837.3**	**1.1**	**0.2**	**0.9**
		Cleaning + deposition	**7**	**6837.7**	**1.4**	**0.1**	**1**
B. arthropoda	SURVIVAL						
		Cleaning	**3**	**244.6**	**0**	**0.5**	**0.5**
		Cleaning *deposition	**5**	**245.9**	**1.3**	**0.3**	**0.8**
		Cleaning + deposition	4	246.7	2.1	0.2	1
		Deposition	3	254.7	10.1	0	1

TABLE A2.2: Results of the semi-parametric model for time-to-event analysis, the Cox proportional hazards model for germination, seedlings emergence and survival of *Castilla ulei* and *Hymenaea parvifolia* with most plausible models according to $\mathrm{AIC_c}$, which are the ones with $\Delta \mathrm{AIC_c}<2$ and the model with fewer variables when more than one had $\Delta \mathrm{AIC_c}<2$. The model effect is the hazard rate (HR) of an event occur.

			HR	Coef.	95% CI	*p*	χ2	*df*
C. ulei	GERMINATION							
		cleaned	15.42	2.74	8.91-26.70	<0.001	95.48	1.00
		frailty(tree)				0.01	10.62	2.80
	SEEDLINGS							
		cleaned	46.08	3.83	16.17-131.29	<0.001	51.43	1.00
		depositionhet	2.71	0.99	0.75-9.69	0.13	2.34	1.00
		cleaned:depositionhet	0.21	-1.58	0.05-0.80	0.02	5.23	1.00
		frailty(tree)				<0.001	87.61	3.87
	SURVIVAL							
		cleaned	0.6	-0.511	0.52-0.70	<0.001	45.51	1.00
		frailty(tree)				0.09	4.57	1.92
H. parvifolia	GERMINATION							
		depositionhet	1.54	0.43	1.03-2.28	0.03	4.54	1.0
		frailty(tree)				0.93	0	0

TABLE A2.3: Results of generalized linear mixed model (GLMM) of *Byrsonima arthropoda*'s survival with variable cleaning (cleaned versus seeds with pulp) as fixed effect and central tree as random effect.

			Estimate	*St. error*	Z	*p*
B. arthropoda	*Fixe effect*	(intercept)	0.51	0.13	3.81	0.000
		cleanseed	-0.65	0.21	-3.11	0.002
			Variance	*Std.dev.*		
	Random effect					
		(intercept)	0.13	0.11		