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Tree recruitment after native frugivore extinction? A field experiment to test the impact of fruit flesh persistence in a tropical oceanic island

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Abstract

The loss of large frugivores leads to seed dispersal loss and regeneration failure of numerous large-seeded trees near mother trees. Although Janzen–Connell effects are considered as the primary underlying cause, other factors remain understudied. Here, we used a field experiment to test the impact of flesh persistence on the recruitment of two large-seeded Sapotaceae species that lost their dispersers. In the rainforest of Mare Longue (Réunion), we sowed 3840 seeds in a four-factor design: seed treatment (seed cleaning; flesh persistence), canopy closure (understorey; gap), year of sowing (01/2018; 11/2019) and species (*Labourdonnaisia calophylloides*, *Mimusops balata*). We also used camera traps to evaluate the impact of extant vertebrates. Seed treatment was by far the most influential factor: flesh persistence led to seedling recruitment divided by 3.2 on average, mainly due to failure of germination or seedling emergence. There were also significant variations in recruitment between species, years and canopy closure levels, notably due to the behaviour of the invasive fauna, especially giant snails that could unexpectedly restore recruitment by feeding on fruit flesh. Together, our results demonstrate strongly depleted recruitment due to flesh persistence and the importance of field experiments to understand the processes at work in complex ecosystems with novel plant–animal interactions.

Introduction

The loss of frugivorous vertebrates leads to disrupted seed dispersal in numerous tropical fleshy-fruited species, among which large-seeded trees are particularly vulnerable (Albert *et al.* 2020b; Effiom *et al.* 2013; Harrison *et al.* 2013; Terborgh *et al.* 2008; Trolliet *et al.* 2019; Vanthomme *et al.* 2010). In turn, this disruption primarily leads to the inability of seeds to move away from mother trees and to the persistence of flesh around seeds because the reward is no longer consumed by frugivorous vertebrates. When both phenomena occur together, unconsumed fruits accumulate at the foot of parent trees where recruitment failure is often observed in tropical forests (see for example Plate 1 in Terborgh *et al.* 2008). To explain the recruitment failure of many tree species that lost dispersal, Janzen–Connell effects have long been put forward as the central mechanism (Comita *et al.* 2014; Connell 1971; Hansen *et al.* 2008; Janzen 1970; Terborgh 2012). However, the large meta-analysis of Rogers *et al.* (2021 PREPRINT) has recently suggested that the absence of seed cleaning alone may have large demographic consequences on fleshy-fruited plant populations at all latitudes, on continents as well as on islands. Several field experiments have shown that flesh persistence strongly decreases the recruitment of fleshy-fruited trees independently of other mechanisms (Fedriani *et al.* 2012; Levi & Peres 2013). Levi and Peres (2013) even suggested that Janzen–Connell effects are a second-order effect acting in numerous tropical plant species once seeds have been successfully cleaned. Apart from these works, field studies investigating the mechanisms involved in recruitment failure as a result of frugivore loss remain scarce to our knowledge.

Flesh persistence may lead to non-innate dormancy, which is the quiescence of seeds that are prevented from germinating only by the unfavourable environment within fleshy fruits (Baskin & Baskin 2004). In other words, the loss of frugivores can theoretically lead to the dormancy of all seeds of certain fleshy-fruited species. Non-innate dormancy may be induced by germination inhibitors (chemical dormancy), low water potential and/or mechanical resistance within the pericarp (or other fleshy appendage such as aril) (Baskin & Baskin 2004). Non-innate dormancy may be very problematic for numerous tropical rainforest trees that often show fast germination and possess recalcitrant seeds, i.e. that have a high moisture content and easily lose their viability because of low tolerance to dehydration (Vazquez-Yanes & Orozco-Segovia 1993). Thus, delaying germination beyond a critical threshold of dryness would lead to lower recruitment for recalcitrant species whose regeneration depends more on seedling stocks in the forest understorey

than on seeds persisting in soil banks (Hopkins & Graham 2006). Finally, although most studies have focused on the role of seed coat scarification (Barnea *et al.* 1991; Soltani *et al.* 2018; Traveset 1998) or even fantasized obligatory plant/seed-coat scarificator interactions (see the criticism of Temple [1977] in Baider & Florens [2006] for the iconic case of the dodo and the tambalacoque tree for instance), non-innate dormancy due to flesh persistence alone may be a major handicap for a large number of fleshy-fruited tree species (Rogers *et al.* 2021 PREPRINT).

A fleshy pericarp primarily rewards frugivores that will help disperse the plant seeds (Eriksson *et al.* 2000; Rick & Bowman 1961; van der Pijl 1982). Although it may protect seeds by deterring seed-eating insects (Silvius & Fragoso 2002), its pervasive persistence due to frugivore loss can also entice natural enemies (Hansen *et al.* 2008; Levi & Peres 2013; Wyse Jackson *et al.* 1988). In Sapotaceae for instance, fungal pathogens may be an important cause of germination failure (Levi & Peres 2013; Wyse Jackson *et al.* 1988). The latter studies, however, do not disentangle whether fungi become pathogenic to seeds that have become dormant due to flesh persistence or whether they merely degrade seeds that are no longer viable. The persistence of flesh may similarly attract seed predators such as rodents in continental tropical forests (Levi & Peres 2013), but also in island forests where they have been introduced and have become invasive since human colonisation (Hansen *et al.* 2008; Shiels & Drake 2011). For example, in Mauritius, Hansen *et al.* (2008) report that ship rats are only interested in diaspores of *Syzygium mamillatum* (Myrtaceae) when seeds have not been hand-cleaned.

Here, we ran a field experiment to test whether flesh persistence decreases the recruitment of seedlings in tropical rainforests on Réunion (Mascarenes). Réunion is a relevant study system of frugivore loss since this oceanic island lost all its native large-bodied frugivores, jeopardising the regeneration of lowland forests (Albert *et al.* 2020b, 2021). To test the impact of flesh persistence, we studied two large-seeded fleshy-fruited tree species, *Labourdonnaisia calophylloides* Bojer and *Mimosa balata* (Aubl.) C.F. Gaertn (Sapotaceae), that lost their native dispersal agents (namely giant tortoises, fruit pigeons, flying-foxes and parrots) in less than two centuries of human colonisation (Albert, 2020). The experiment was realised in the absence of conspecific trees nearby, to avoid possible Janzen–Connell effects. Because seeds should normally be dispersed in different spatio-temporal contexts, we tested the effect of seed cleaning under two canopy closure levels and two years apart. Finally, we monitored the interactions with introduced animals, particularly ship rats *Rattus rattus* and giant snails *Lissachatina immaculata* known for their potential negative impact on fruits, seeds or seedlings (Bissessur *et al.* 2020; Meyer & Shiels 2009; Shiels & Drake 2011).

Study site and focal species

Located 750 km East of Madagascar and 230 km West-West-South of Mauritius, Réunion is the largest (2512 km²) and highest (3070 m) island of the Mascarenes. The study site was located in the South-East of Réunion on the wet windward side at Mare Longue (mean annual precipitation: ca 4300 mm, Réchou *et al.* 2019) which, at the ecozone scale, hosts one of the last corridors of native forest from the coastal area to the subalpine shrubland (Figure 1). The lowland tropical rainforest of Mare Longue mainly grows on a recent pāhoehoe lava flow (age = ca 430 years old) displaying a very thin soil on continuous rock (Meunier *et al.* 2010), which probably explains why this area has not been converted to

agriculture (Albert *et al.* 2020a). Although fairly recent, the native plant community assembly at Mare Longue occurred before the beginning of human colonisation and the rapid extirpation of all large native frugivores (Albert *et al.* 2020b). With more than 40 tree species per ha and trees exceeding 20 m in height, this lowland forest is thus one of the best preserved in the Mascarenes (Cadet 1977; Strasberg 1996). At low elevation, the forest corridor is composed of sixty-eight hectares of pristine native forest that were early protected in a national nature reserve in the 1980s and are now part of a larger national park. The pristine forest fragments are embedded in a matrix of secondary forest, quite heterogeneous regarding vegetation types, with native species-rich habitats alternating with plantations of native trees and invaded areas.

Our field experiment took place at Ilet Tesson (Figure 1) with no conspecific mother trees nearby and therefore minimal influence of exogenous seeds and natural enemies of focal trees. We also chose sowing areas to avoid temporary streams that occur during heavy rain events and could strongly impact the distribution of seeds and fruits within the experimental design. Sowing areas were a maximum of 50 m apart. Understory situations initially showed a shrub stratum dominated by *Syzygium jambos* (Myrtaceae), a highly invasive species on Réunion (Macdonald *et al.* 1991). Sowing areas in canopy gaps were invaded mostly by the exotic shrub *Clidemia hirta* (Melastomataceae) (Mandon-Dalger *et al.* 2004). Both invasive species were uprooted before sowing and controlled all along the experiment.

The focal species are large-seeded (mean seed diameter of 12 mm in *L. calophylloides* and 24 mm in *M. balata*) and typical species of the Sapotaceae family that grow as long-lived shade-tolerant trees in mature tropical rainforests (Levi & Peres 2013; Mahandran *et al.* 2018). These trees are often dominant in native lowland canopies and not threatened like other large-seeded Sapotaceae in the Mascarenes (Baider & Florens 2006; Cadet 1977; Strasberg 1996), despite their incapacity to recolonize the vast areas of historical lava flows due to frugivore loss (Albert *et al.* 2020b). Both species have been massively planted by the French National Office in the area of Saint-Philippe. In old-growth forests, *L. calophylloides* sometimes regenerates very well and produces dense seedling banks, but the situation is often much more contrasted for *M. balata* (Albert *et al.* 2021). Thus, if our field experiment were to show a strong negative impact of flesh persistence on tree recruitment, corroborating the work of Levi & Peres (2013), it is that other factors favour the recruitment of these two focal species in tropical rainforest despite the loss of all large native frugivores on Réunion.

Methods

Experimental design

We collected freshly fallen ripe fruits of *L. calophylloides* and of *M. balata* at the foot of parent trees in the forest of Mare Longue in late December 2017 and November 2019. For each species, we hand-cleaned 500 seeds and kept an equivalent number of seeds within intact fruits: 500 fruits for the single-seeded *L. calophylloides* and for *M. balata*, 168 fruits in January 2018 (mean of 2,9 seeds per fruit) and 250 fruits in November 2019 (mean of 2 seeds per fruit). By hand-cleaning, we mean removing the whole pericarp and rinsing the seeds to remove residual debris, without scarification. This task was particularly tedious for *L. calophylloides* whose latex-rich pericarp must be extracted with warm water. Fruits and seeds were stored in a cold room for three weeks maximum before sowing.

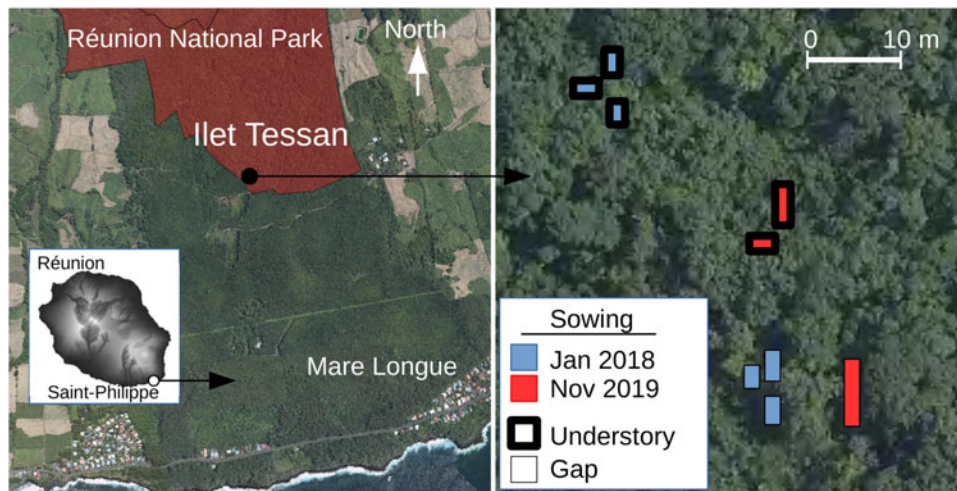


Figure 1. Study site. The forest corridor of Mare Longue, municipality of Saint-Philippe, is the most preserved corridor of native forests from sea level to high elevations (>2200 m asl) in the Mascarenes. The field experiment is located at Ilet Tesson around 150 m asl, at the edge of the Réunion National Park. In order to adapt to rugged terrain on a recent pāhoehoe lava flow, quadrats of varying size were delineated for each of the four sowing areas (combination of factors 'date of sowing' & 'canopy closure'). In each sowing area and for both focal species, *Labourdonnaisia calophylloides* and *Mimusops balata*, 'hand cleaning of seeds' and 'flesh persistence' modalities were replicated 12 times at a rate of 20 seeds per modality. For a detailed view of the sowing, please report to Appendix 1.

Ex situ tests

To test whether there was an intrinsic limitation prior to the field experiment, we hand-cleaned 500 additional seeds in January 2018 and realised viability and germination tests. We used the colorimetric test of tetrazolium chloride (0,1 % i.e. 0,1 g of powder/100 mL of filtered water), which leads to a carmine red colouration of the living embryos after six hours of soaking (Porter *et al.* 1947). We performed five repetitions on 20 seeds for each viability test. Seeds of *L. calophylloides* and *M. balata* showed a viability of, respectively, $93\% \pm 2,7$ SD and $86\% \pm 4,2$ SD. These results indicated that seeds used for the experiment showed a very high viability rate.

Field experiment

The effect of seed cleaning vs flesh persistence was tested under two levels of canopy closure, resulting in four combinations with 12 repetitions per combination. At the rate of 20 seeds per repetition, we thus sowed a total of 3840 seeds: 960 seeds for each species both in January 2018 and in November 2019. The experimental design was materialised with wire mesh on the ground, each repetition being represented as a $0,4 \times 0,4$ m quadrat (Supplementary material, Appendix 1).

Rigorous evaluation of germination in this experiment was not possible because early stages of root development could not be observed when it took place within the fruit (Figure 2). We monitored seedling recruitment by counting the number of rooted seedlings after emergence. Réunion was subject to several tropical depressions during the first months of the experiment in 2018 (Berguitta and Dumazile cyclones in January and March 2018, respectively), we therefore monitored the number of fruits and seeds after cyclones, which was made possible thanks to the large size of focal species seeds (Figure 2 & Appendix 1). These cyclones had no impact on the experimental design.

Seed and fruit predation by *R. rattus* has already been reported for Sapotaceae seeds in the Mascarenes (Baider & Florens, 2006; Eric Rivière, pers. comm.), that is why we took into account the potential impact of this introduced species. Due to the difficulty in setting up predator exclosures on rough substrates like those at Ilet Tesson (Appendix 2), we monitored seed removal by counting the number of seeds and fruits in each replicate repeatedly during six months after sowing. We also used two Bushnell Trophy Cam HD to monitor potential seed and fruit predators from January 2018 to January 2020. These digital camera traps were



Figure 2. Germination of *Mimusops balata* (above) and *Labourdonnaisia calophylloides* (below), photo taken five weeks after sowing. Here, i.e. 'hand-cleaning of seeds' modality, germination can be seen, but in the case of 'flesh persistence', only the emergence of seedlings from the fruit can be properly assessed. Photos: A. Gorissen

set up in the understory about 1,5 metre from sown seeds and fruits and set to operate day and night with movement triggers.

After sowing in January 2018, temporal monitoring of seedling emergence was carried out approximately every 15 days for 8 months, then once a month thereafter. For the second sowing in November 2019, monitoring of seedling emergence could only be performed once, 6,5 months after sowing due to logistic restrictions, that is, when almost all seedling emergences had occurred.

Statistical analyses

Seedling recruitment was calculated as the proportion of emerged/rooted seedlings relative to the number of unremoved or

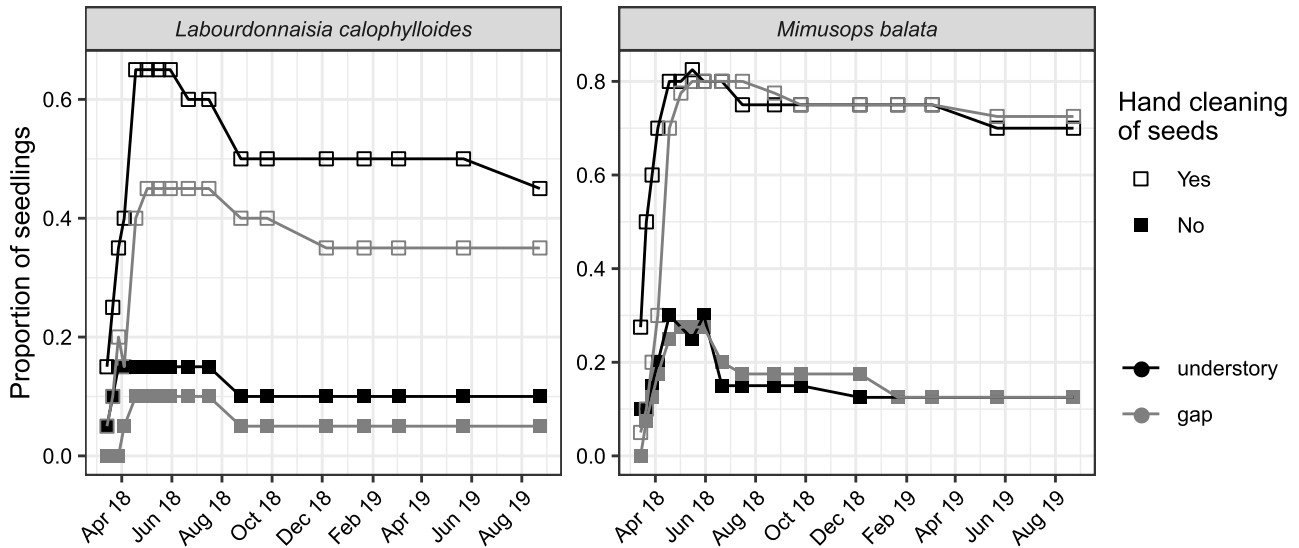


Figure 3. Temporal monitoring of seedling recruitment of *Labourdonnaisia calophylloides* and *Mimusops balata* after the first sowing in January 2018. Seedling recruitment is calculated as the proportion of emerged/rooted seedlings relative to the number of unremoved or unpredated seeds. For each date, squares display the median values. Full and empty squares display ‘flesh persistence’ and ‘hand cleaning of seeds’ modalities, respectively; black and grey squares display ‘understory’ and ‘canopy gap’ modalities, respectively.

unpredated seeds (data set available as [Supplementary material II](#)). We modelled seedling recruitment depending on four categorical variables with two levels: seed treatment (hand cleaning *versus* flesh persistence), canopy closure (canopy gap *versus* understory), date of sowing (January 2018 *versus* November 2019) and species (*L. calophylloides* *versus* *M. balata*) using a binomial generalised linear model (GLM) with a logit link. We tested all possible models with four explicative variables as well as their two-way interactions and eventually retained the model that displayed the lowest AIC.

Results

Temporal monitoring confirmed the emergence of seedlings as soon as two months after sowing in January 2018 (Figure 3). Emergence was especially frequent in hand-cleaned seeds for which median proportions peaked in May 2018. Field observations showed that the emergence of all seedlings occurred within the first six months after sowing. Seedling survival was relatively high afterwards despite an unusual drought event that struck the area between December 2018 and April 2019. Seedling recruitment subsequent to the two sowing periods was compared at 6.5 months.

The best fitted GLM included the four factors (‘seed treatment’, ‘canopy closure’, ‘date of sowing’, ‘species’) with all possible two-way interactions ($\text{pseudo-}R^2_{\text{glm}} = 0,697$, Appendix 3). Seed treatment was by far the best predictors (Appendix 4), flesh persistence having a strong negative impact on estimated seedling recruitment ($1/3,2$, Figure 4). Field observations showed that uncleaned seeds of *L. calophylloides* often remained trapped within the persisting pericarp (Appendix 5). Likewise, germination of uncleaned seeds often occurred in *M. balata*, but seedlings had difficulties rooting in the soil due to the thickness of the exocarp. Also the first roots remained exposed to open air after flesh disappearance (Appendix 6). The instability of the exocarp that generally persisted after the disappearance of the mesocarp often resulted in tilting movements of seedlings (Appendix 6). The positive impact of hand cleaning of seeds was stronger in the first year of the experiment, as illustrated by the highly significant interaction between both factors (Figure 4 & Appendix 4).

In the second year, the effect of hand cleaning was mitigated in the understory where *Lissachatina immaculata* fed heavily on fruits of focal species (especially *L. calophylloides*, Appendices 7 & 8), but it remained strong in the canopy gap where few *Lissachatina immaculata* were observed. Over the two years of the experiment, *M. balata* showed overall higher estimated recruitment than *L. calophylloides* (0,46 *versus* 0,37) and a stronger positive effect of hand cleaning of seeds ($\times 3,7$ *versus* $\times 2,4$) resulting in higher recruitment rates for *M. balata* (0,72) than for *L. calophylloides* (0,52) (Figure 4). Finally, *M. balata* showed a higher recruitment than *L. calophylloides* in the canopy gaps.

In 2018, we did not detect any noticeable impact of introduced vertebrates in terms of fruit or seed removal of focal species. The same year, however, observations by camera traps showed high levels of fruit or seed removal by *R. rattus* on other native tree species at Ilet Tesson (Appendix 7). In November 2019, 22 fruits of *L. calophylloides* and four seeds of *M. balata* were removed and predated by *R. rattus*, respectively. Likewise, although snails were seen eating the flesh of focal species in January 2018, the phenomenon was much more frequent in November 2019, and the giant snail *Lissachatina immaculata* sometimes performed very effective seed cleaning especially in the understory (for details on the other animals monitored at Ilet Tesson, see Appendix 7).

Discussion

Most studies exploring the consequences of frugivore loss on tree recruitment focused on the Janzen–Connell hypothesis (Comita et al. 2014; Hansen et al. 2008; Terborgh 2012; Terborgh et al. 2008). In contrast, few field studies have investigated the impact of pervasive flesh persistence around seeds that results from the non-consumption of fruits after frugivores have been extirpated. The present field experiment confirms that flesh persistence may be a primary cause of recruitment loss of the two focal large-seeded Sapotaceae trees (*L. calophylloides* and *M. balata*). Our results corroborate other field studies in the Neotropics (Levi & Peres 2013) and in the mediterranean area (Fedriani et al. 2012) and are also in accordance with numerous

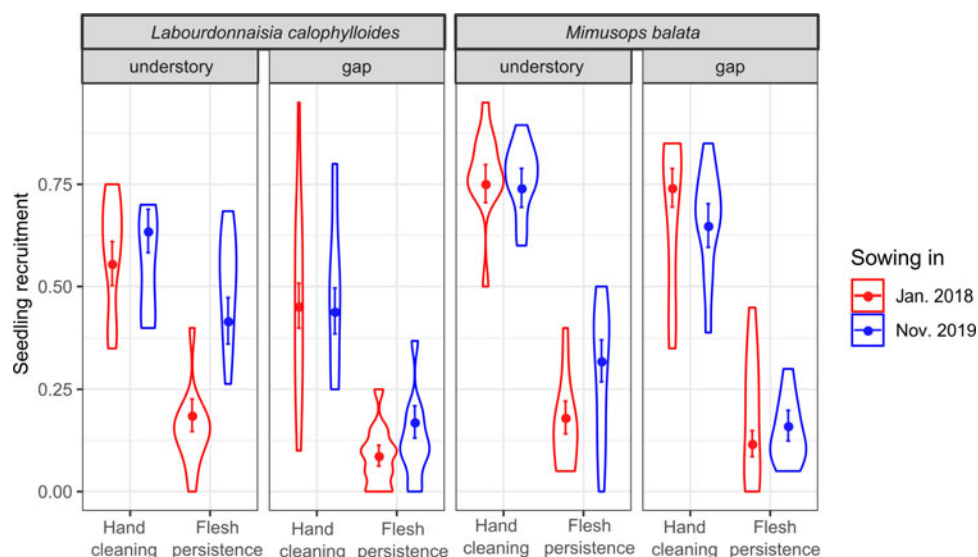


Figure 4. Seedling recruitment of *Labourdonnaisia calophylloides* and *Mimusops balata* 6.5 months after sowing. Seedling recruitment is calculated as the proportion of emerged seedlings relative to the number of unremoved or unpredated seeds. Violin plots show raw data; points and error bars display mean values and confidence intervals predicted by the best-fitting GLM, respectively.

studies carried out under controlled conditions (Traveset 1998, Rogers *et al.* 2021 PREPRINT). Together, this demonstrates that the negative impact of flesh persistence has probably been underestimated to date. This also shows that the fleshy reward that has been selected to effectively disperse large seeds in a highly competitive environment (Eriksson *et al.* 2000; but see Silvius & Fragoso 2002) may alone become a major handicap for large-seeded plants when mutualist partners are extirpated. Lastly, our study shows significant inter-annual fluctuations in recruitment resulting from the complex role played by invasive animals, which emphasises the difficulty of understanding the consequences of native frugivore extinction in island ecosystems with novel plant–animal interactions (Meyer & Shiels 2009; Shiels & Drake 2011; Vizentin-Bugoni *et al.* 2019).

Mechanisms by which flesh persistence may strongly limit recruitment

Several mechanisms may be involved in the negative effect of flesh persistence on tree recruitment. We distinguish here between those which act before and after germination. Before germination, our results suggest that flesh persistence induces a non-innate dormancy in focal species (Baskin & Baskin 2004). Indeed, the hardness of the exocarp, especially in *M. balata* (Appendix 6) and the presence of hydrophobic compounds in the latex-rich flesh of focal species (Appendix 5), might lead to a decrease in water level, preventing the induction of germination. Likewise, germination might be directly prevented by chemical dormancy or inhibited by mechanical resistance whatever the water potential (Baskin & Baskin 2004). Germination failure might also be due to the action of pathogenic microorganisms, as suggested by Wyse Jackson *et al.* (1988) and noted by Levi and Peres (2013) in the same botanical family. Further investigation is needed to unravel the mechanisms at this stage. After germination, we show that flesh persistence may limit the survival of seedlings. The hardness of the exocarp results in a major handicap for many seedlings because the root system not only has difficulty crossing the exocarp but also tends to develop in the flesh (Appendix 6). The roots of many seedlings thus remain in the open air once the flesh has disappeared. These seedlings are subsequently poorly anchored and vulnerable to biotic and abiotic hazards in the understory. As simple as this may seem,

we did not find any studies documenting this type of recruitment limitation.

The negative impact on recruitment and the relative commonness of focal species: a paradox?

Focal species have been chosen also because they produce a large amount of fruit almost every year. Although these species have not been able to recolonize historical lava flows of the *Piton de la Fournaise* due to strong dispersal limitation (Albert *et al.* 2020b), they reach high levels of recruitment locally in old-growth forests, especially *L. calophylloides* which is dominant at all stages of growth in permanent plots in Mare Longue forest for instance (Albert *et al.* 2021; Strasberg 1996). We suspect that the local dominance of *L. calophylloides* might be due to the recruitment failure of other large-fleshy-fruited canopy species leading to lower alpha-diversity and community evenness (Albert *et al.* 2021). Given the strong recruitment limitation induced by flesh persistence in our experiment, then how to explain such a paradox? First of all, emergence rates of about 10% without seed cleaning might be enough to sustain populations where tree fecundity is high. Second, as an alternative (or complementary) hypothesis, the ability of several introduced animals to modulate the recruitment in our experiment suggests that focal species may have benefited from novel and complex interactions since the extirpation of native frugivores, as discussed hereafter and documented elsewhere (Loayza *et al.* 2020; Shiels & Drake 2011).

At Ilet Tesson, invasive giant snails *Lissachatina immaculata* usually known for their negative impact on native plants on islands (Tillier 1992) can partially support the recruitment of *L. calophylloides* by cleaning numerous seeds (Appendix 8). While the role of giant snails should not be overestimated because they do not have the capacity to move away large seeds from mother trees, they are usually seen feeding on large fleshy fruits of other tree species and might consequently favour their recruitment in the understory of old-growth forests. Also their impact on seedlings as herbivores might outweigh the benefits of seed cleaning, although we did not observe any browsing during our experiment.

R. rattus actually plays an ambivalent role as seed predator and secondary disperser (Shiels & Drake 2011). In our case, fruits and seeds of focal species were not attractive in 2018, unlike some other

fleshy-fruited plants such as *Hyophorbe indica* the same year (Appendix 7). In November 2019, rats undeniably destroyed some seeds of *M. balata* as already shown for other large-seeded plants on Réunion (Albert 2020), but behaved mainly as secondary dispersers of *L. calophylloides*. Doing so, one might argue that they significantly contribute to the recruitment of this tree. However, *R. rattus* is probably a poor disperser because fruits are usually stored in trees and seeds remain in the flesh of wasted fruits (Sébastien Albert, pers. obs.).

Poachers have been hunting birds with lime sticks charged with glue made from the latex of focal species (Cheke 1987). Field observations show numerous seedlings of *L. calophylloides* near areas where poachers remove the flesh from fruits in old-growth forests. Until recently, this type of poaching was a widely practised tradition (Cheke 1987), so poachers might have dispersed numerous seeds of this focal species for centuries.

Regarding the other focal species, *M. balata*, the situation is more contrasted in terms of sapling recruitment. For example, this species does not regenerate well in the two best-preserved permanent plots where large mother trees are encountered (Albert et al. 2021; Strasberg 1996). One may argue that this species is more light demanding than *L. calophylloides* which ultimately explains this lack of recruitment (Debroize 2003). Although the present experiment shows that this species also regenerates very well in the understory once the seeds have been hand cleaned, more time may be needed to detect seedling/sapling dieback in the understory. Since *M. balata* has the largest fruit in the native flora, the loss of native frugivores may have led to its pervasive inability to reach gaps where light conditions are favourable. Ongoing field experiments on Réunion should provide information in the coming years.

Lastly, our field experiment shows that large-seeded trees can reach high recruitment rates in previously weeded areas notwithstanding the hyperskeletal soil (Appendix 2). If these recalcitrant species are quickly sown, seedling recruitment rates in the field can be as high as germination rates in *ex situ* nursery reported by Debroize (2003). Thus, combining invasive species control with direct sowing is relevant for considering restoration actions in the long term (Appendix 9), especially since native tree species that are still dispersed will probably benefit from alien plant weeding (Baider & Florens 2011).

Supplementary material. To view supplementary material for this article, please visit <https://doi.org/10.1017/S026646742200027X>

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Conflicts of interest. The authors declare none.

References

- Albert S (2020) *Rupture des interactions mutualistes plantes à fruits charnus-vertébrés frugivores, et conséquences sur la régénération des forêts tropicales dans les Mascareignes*. Doctoral dissertation. Université de La Réunion.
- Albert S, Flores O, Baider C, Florens FBV and Strasberg D (2021) Differing severity of frugivore loss contrasts the fate of native forests

- on the land of the Dodo (Mascarene archipelago). *Biological Conservation* 257, 109131.
- Albert S, Flores O, Michon L and Strasberg D (2020a) Dating young (<1000 yr) lava flow eruptions of Piton de la Fournaise volcano from size distribution of long-lived pioneer trees. *Journal of Volcanology and Geothermal Research* 401, 106974.
- Albert S, Flores O and Strasberg D (2020b) Collapse of dispersal trait diversity across a long-term chronosequence reveals a strong negative impact of frugivore extinctions on forest resilience. *Journal of Ecology* 108, 1386–1397.
- Baider C and Florens FBV (2006) *Current decline of the 'Dodo-tree': a case of broken-down interactions with extinct species or the result of new interactions with alien invaders*. In Laurance W and Peres CA (eds.), *Emerging Threats to Tropical Forests*, Chicago: Chicago University Press, pp. 199–214.
- Baider C and Florens FBV (2011) Control of invasive alien weeds averts imminent plant extinction. *Biological Invasions* 13, 2641–2646.
- Barnea A, Yom-Tov Y and Friedman J (1991) Does ingestion by birds affect seed germination? *Functional Ecology* 5, 394.
- Baskin JM and Baskin CC (2004) A classification system for seed dormancy. *Seed Science Research* 14, 1–16.
- Bissessur P, Baider C and Florens FBV (2020) Infestation by pollination-disrupting alien ants varies temporally and spatially and is worsened by alien plant invasion. *Biological Invasions* 22, 2573–2585.
- Cadet T (1977) *La végétation de l'île de la Réunion: étude phytoécologique et phytosociologique* (Doctoral dissertation), Université d'Aix-Marseille.
- Cheke AS (1987) *The ecology of the surviving native land-birds of Reunion*. In Diamond AW (ed.), *Studies of Mascarene Island Birds*. Cambridge: Cambridge University Press, pp. 301–358.
- Comita LS, Queenborough SA, Murphy SJ, . . . Zhu Y (2014) 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* 102, 845–856.
- Connell JH (1971) On the role of natural enemies in preventing competitive exclusion in some marine animals and in rain forest trees. In Den Boer PJ and Gradwell GR (eds), *Dynamics of Populations*, 298, 298–312.
- Debroize N (2003) *Le grand natte (Mimops maxima) en forêt mégatherme hygrophile à l'île de la Réunion*. Montpellier: ENGREF, 76 p. Mémoire: Ecole nationale du génie rural, des eaux et des forêts.
- Effiom EO, Nunez-Iturri G, Smith HG, Ottosson U and Olsson O (2013) Bushmeat hunting changes regeneration of African rainforests. *Proceedings of the Royal Society B: Biological Sciences* 280, 20130246.
- Eriksson O, Friis EM and Löfgren P (2000) Seed size, fruit size, and dispersal systems in angiosperms from the early Cretaceous to the late Tertiary. *The American Naturalist* 156, 47–58.
- Fedriani JM, Zywiec M and Delibes M (2012) Thieves or mutualists? Pulp feeders enhance endozoochore local recruitment. *Ecology* 93, 575–587.
- Hansen DM, Kaiser CN and Müller CB (2008) Seed dispersal and establishment of endangered plants on oceanic islands: the Janzen-Connell model, and the use of ecological analogues. *PLoS ONE* 3, e2111.
- Harrison RD, Tan S, Plotkin JB . . . Davies SJ (2013) Consequences of defaunation for a tropical tree community. *Ecology Letters* 16, 687–694.
- Hopkins M and Graham AW (2006) The viability of seeds of rainforest species after experimental soil burials under tropical wet lowland forest in north-eastern Australia. *Australian Journal of Ecology* 12, 97–108.
- Janzen DH (1970) Herbivores and the number of tree species in tropical forests. *American Naturalist* 104, 501–528.
- Levi T and Peres CA (2013) Dispersal vacuum in the seedling recruitment of a primate-dispersed Amazonian tree. *Biological Conservation* 163, 99–106.
- Loayza AP, Luna CA and Calviño-Cancela M (2020) Predators and dispersers: context-dependent outcomes of the interactions between rodents and a megafaunal fruit plant. *Scientific Reports* 10, 6106.
- Macdonald IAW, Thébaud C, Strahm WA and Strasberg D (1991) Effects of alien plant invasions on native vegetation remnants on La Réunion (Mascarene Islands, Indian Ocean). *Environmental Conservation* 18, 51–61.
- Mahandran V, Murugan CM, Marimuthu G and Nathan PT (2018) Seed dispersal of a tropical deciduous Mahua tree, *Madhuca latifolia* (Sapotaceae) exhibiting bat-fruit syndrome by pteropodid bats. *Global Ecology and Conservation* 14, e00396.

- Mandon-Dalger I, Clergeau P, Tassin J, Rivière J-N and Gatti S** (2004) Relationships between alien plants and an alien bird species on Reunion Island. *Journal of Tropical Ecology* **20**, 635–642.
- Meunier JD, Kirman S, Strasberg D, Nicolini E, Delcher E and Keller C** (2010) The output and bio-cycling of Si in a tropical rain forest developed on young basalt flows (La Reunion Island). *Geoderma* **159**, 431–439.
- Meyer WM and Shiels AB** (2009) Black rat (*Rattus rattus*) predation on non-indigenous snails in Hawai'i: complex management implications. *Pacific Science* **63**, 339–347.
- Porter RH, Durrel M and Romm HJ** (1947) The use of 2, 3, 5-triphenyl-tetrazolium chloride as a measure of seed germinability. *Plant Physiology* **22**, 149–159.
- Réchou A, Flores O, Jumeaux G . . . Bonnardot F** (2019) Spatio-temporal variability of rainfall in a high tropical island: patterns and large-scale drivers in Réunion Island. *Quarterly Journal of the Royal Meteorological Society* **145**, 893–909.
- Rick CM and Bowman RI** (1961) Galapagos tomatoes and tortoises. *Evolution* **15**, 407–417.
- Rogers HS, Cavazos BR, Gawel AM, Karnish A, Ray CA, Rose E, Thierry H and Fricke EC** (2021 PREPRINT). Frugivore gut passage increases seed germination: an updated meta-analysis. Preprint at <http://biorxiv.org/lookup/doi/10.1101/2021.10.12.462022>.
- Shiels AB and Drake DR** (2011) Are introduced rats (*Rattus rattus*) both seed predators and dispersers in Hawaii? *Biological Invasions* **13**, 883–894.
- Silvius KM and Fragoso JMV** (2002) Pulp handling by vertebrate seed dispersers increases palm seed predation by Bruchid beetles in the northern Amazon. *Journal of Ecology* **90**, 1024–1032.
- Soltani E, Baskin CC, Baskin JM, Heshmati S and Mirfazeli MS** (2018) A meta-analysis of the effects of frugivory (endozoochory) on seed germination: role of seed size and kind of dormancy. *Plant Ecology* **219**, 1283–1294.
- Strasberg D** (1996) Diversity, size composition and spatial aggregation among trees on a 1-ha rain forest plot at La Réunion. *Biodiversity & Conservation* **5**, 825–840.
- Temple SA** (1977) Plant-animal mutualism: coevolution with dodo leads to near extinction of plant. *Science* **197**, 885–886.
- Terborgh J** (2012) Enemies maintain hyperdiverse tropical forests. *The American Naturalist* **179**, 303–314.
- Terborgh J, Nuñez-Iturri G, Pitman NCA . . . Paine CET** (2008) Tree recruitment in an empty forest. *Ecology* **89**, 1757–1768.
- Tillier S** (1992) Introduced land snails in New Caledonia: a limited impact in the past, a potential disaster in the future. *Pacific Science* **46**, 396–397.
- Traveset A** (1998) Effect of seed passage through vertebrate frugivores' guts on germination: a review. *Perspectives in Plant Ecology, Evolution and Systematics* **1**, 151–190.
- Trollet F, Bauman D, Forget P-M, Doucet J-L, Gillet J-F and Hambuckers A** (2019) How complementary are large frugivores for tree seedling recruitment? A case study in the Congo Basin. *Journal of Tropical Ecology* **35**, 223–236.
- van der Pijl L** (1982) *Principles of Dispersal in Higher Plants, Third Revised and Expanded Edition*. Berlin Heidelberg, New York: Springer Verlag.
- Vanthomme H, Bellé B and Forget P-M** (2010) Bushmeat hunting alters recruitment of large-seeded plant species in Central Africa. *Biotropica* **42**, 672–679.
- Vazquez-Yanes C and Orozco-Segovia A** (1993) Patterns of seed longevity and germination in the tropical rainforest. *Annual Review of Ecology and Systematics* **24**, 69–87.
- Vizentin-Bugoni J, Tarwater CE, Foster JT . . . Sperry JH** (2019) Structure, spatial dynamics, and stability of novel seed dispersal mutualistic networks in Hawai'i. *Science* **364**, 78–82.
- Wyse Jackson PS, Cronk QCB and Parnell JAN** (1988) Notes on the regeneration of two rare Mauritian endemic trees. *Tropical Ecology* **29**, 98–106.