

EFFECTS OF MANAKIN GUT PASSAGE ON GERMINATION SUCCESS
OF A NEOTROPICAL MELASTOME

A MASTER'S THESIS BY:
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ABSTRACT

As neotropical rainforests become increasingly deforested and fragmented, a better understanding of the influence of frugivore-mediated seed dispersal on regeneration processes is necessary. However, this remains an elusive goal, in part due to the complexity and variety of abiotic and biotic factors (i.e., microsite quality of the deposited seed and/or gut-passage effects from the frugivore) that differentially influence the survival and germination success of dispersed seeds. The ecological relationship between manakins (Aves: Pipridae) and the plant family Melastomataceae represents a classic example of a plant-frugivore relationship in the neotropics. Despite the prevalence of manakins in tropical rainforest, little is known about their ecological contributions via endozoochorous seed dispersal. How manakin gut passage influences the germination of one widely distributed and ecologically important genus of melastomes, positively photoblastic *Miconia*, is unknown. Previous studies investigating how gut passage affects the germination success of melastomes have widely varying results, and, in particular, whether scarification of the seed coat during gut passage influences the germination success of melastomes has not been thoroughly investigated. In this study, we tested how gut passage by manakins in northwestern Ecuador affects the germination of a gap-dependent *Miconia* shrub by conducting a paired germination experiment. Overall, our results suggest that gut passage by manakins accelerate the germination of seeds from this *Miconia* species, especially when passed by *Lepidothrix coronata*. While final germination success (at 80 days) did not differ between treatments, gut-passed seeds had significantly higher mean germination success at earlier time points (i.e., 20 and 40 days) relative to control seeds. Overall, gut-passed seeds had significantly earlier mean germination times than non-digested seeds, although the variance in germination times did not significantly differ between treatments. These results suggest that manakin gut passage accelerates the germination of *Miconia* seeds, likely via mild to moderate scarification of the seed coat. Since manakins are abundant in disturbed habitats, these birds may act as agents of restoration by providing a competitive advantage to gap-dependent pioneer melastomes that likely benefit from earlier germination when deposited in fragmented tropical areas.

INTRODUCTION

The recovery of biodiversity in regenerating habitats may at least partially depend on the delivery of seeds from off-site locations by animal seed dispersers (Duncan & Chapman, 1999; Pejchar et al., 2008; Carlos & Morales, 2016). Because an estimated 75% of neotropical tree species produce fruit eaten by birds, avian frugivores can be considered pivotal in shaping the ecological composition of regenerating neotropical forest (Wenny & Levey, 1998). Birds in particular can act as ecosystem engineers by moving digested seeds large distances from parent plants, potentially into areas undergoing succession where seed deposition is especially crucial (Murray, 1988; Loiselle & Blake, 1993; Sekercioglu, 2012). Seed dispersal can also benefit plants by reducing intraspecific competition and increasing escape from predators and pathogens that concentrate near parent plants (Janzen, 1970; Connell, 1971). However, the benefit of transport of seeds by frugivores to a new location is dependent on environmental determinants of seed fate, such as the microsite quality of the seed's new location (Howe & Smallwood, 1982; Jordano, 2000; Wenny, 2001). In addition, biotic factors, such as gut-passage effects, are also widely regarded as ecologically influential due to how digestive processes from endozoochory can significantly enhance or inhibit germination (Traveset et al., 2007).

Digestive processing of a seed during gut passage by a frugivore can critically affect seed fate via several important mechanisms. First, gut passage may enhance seed germination via depulping, or the removal of the fruity pulp from around a seed during the digestive process. Depulping separates the seed from germination-inhibiting compounds present in fruit pulp and significantly reduces the likelihood of pathogen infestation (i.e., the "deinhibition effect"; Traveset & Verdú, 2002; Samuels & Levey, 2005; Robertson, 2005; Silveira et al., 2012; Ribeiro et al., 2016; Messeder et al., 2022). Second, gut passage may increase germination success if the surrounding fecal matter provides nutrients to the defecated seed (i.e., the "fertilization effect"; Traveset & Verdú, 2002; Robertson, 2005). Third, the handling of a seed prior to

or during ingestion has the potential to damage a seed as it enters the alimentary canal, thereby impacting germination success (Moermond & Denslow, 1985; Levey, 1987). For instance, birds referred to as “gulpers” (e.g., manakins, thrushes, and toucans) swallow fruits whole with minimal handling, while “mashers” (e.g., tanagers, finches, and parrots) more thoroughly manipulate or entirely crush fruits in the bill, the latter being associated with seed death (Janzen, 1981; Levey, 1987; Murray, 1988). Finally, abrasion of the seed coat through the mechanical and chemical handling in the gut can positively or negatively affect germination (i.e., the “scarification effect”; Barnea et al., 1990; Traveset, 1998).

Of the above gut-passage effects, scarification remains the most poorly understood. The primary mechanism by which the scarification effect is thought to enhance germination is by the reduction of the seed coat increasing the seeds’ permeability to water and nutrients (Barnea, 1990; Traveset et al., 2001), although it has also been suggested to increase seeds’ ability to detect light and alter seed dormancy (Murray, 1988; Traveset et al., 2001). Therefore, the scarification effect could have especially important implications on the germination of photoblastic pioneer species, though this has not been thoroughly investigated. Alternatively, scarification can have negative effects on seed fate in association with longer gut-retention times, as an increased amount of handling time in the digestive tract can fatally deteriorate a seed, killing the seed embryo (Murray et al., 1994; Charalambidou et al., 2003). However, other studies have found the opposite, with longer gut-passage times resulting in higher germination success due to more extensive scarification effects (Barnea, 1991). Overall, studies investigating the scarification effect alone have yielded mixed results, therefore the effects of scarification on seed fate is likely highly dependent on the morphology of the seed being consumed and the digestive anatomy of the bird consuming it (Traveset, 1998).

Here, we investigated the influence of gut passage on seed germination in an iconic neotropical fruit-frugivore system: manakin birds (Pipridae) and melastome plants (Melastomataceae), in a heavily agriculturally fragmented tropical rainforest in northwestern Ecuador. Melastomataceae is a widely distributed component of the Neotropical flora and plays a key role in recolonizing disturbed habitats (Ellison et al., 1993; Pearson et al., 2002; Silveira et al., 2013). Manakins are small, lekking passerines that constitute some of the most abundant frugivores in South and Central American rainforests, making them prominent agents of endozoochorous seed dispersal in these habitats (Cestari & Pizo, 2012, 2013). Despite being generalist frugivores, some manakins (e.g., the White-bearded Manakin, *Manacus manacus*) appear to be particularly reliant on melastomes, with the peak breeding season of some species coinciding with peak fruit production by melastomes (Snow, 1962; Krijger et al., 1997). Prior studies of avian gut passage effects in melastomes have yielded widely varied results, with some finding negative, null, or positive effects on germination (Ellison et al., 1993; Alves et al., 2008; Gomes et al., 2008; Silveira et al., 2012; Lessa et al., 2013; Silveira et al., 2013; Ribeiro et al., 2016; Lessa et al., 2019). Importantly, many of these germination experiments have shown gut passage to have a greater effect on germination times than the germinability of seeds, although these effects vary between species and lifeforms (Ellison et al., 1993; Lessa et al., 2013; Silveira et al., 2013). Given the prevalent and close relationship between manakins and melastomes in Neotropical forests, determining the quality of the seed dispersal services that manakins provide to Melastomataceae species is important in the context of restoration ecology in that manakins could behave as ecosystem engineers via dispersing gut-passed seeds of pioneer melastome species into regenerating tropical rainforest.

To determine the influence of gut passage on seed germination in this system, we conducted a germination experiment in which we compared the germination rate of melastome seeds (*Miconia* sp.) passed by manakins to that of control seeds that were manually extracted from fruits. Notably, because seeds were extracted from fruit pulp and/or fecal matter, we were primarily testing the influence of scarification by manakin gut passage. Given that manakins are “gulpers” with rapid gut-passage times (Worthington, 1982; Blake & Loiselle, 2002; Snow, 2004), we hypothesized that manakin gut passage would enhance and accelerate germination of *Miconia* seeds via mild to moderate scarification of the seed coat. In addition, given the relatively similar digestive morphologies across manakins, we did not expect to observe significant differences in gut-passage effects between manakin species or sexes (Worthington, 1982). Examining how gut passage by manakins influences the germination success of an abundant *Miconia*

pioneer aids our understanding in how these frugivores may act as ecosystem engineers and play a part in regeneration of these fragmented habitats.

METHODS

Study Area

This study took place in the Chocó Biogeographic zone of northwestern Ecuador, a global hotspot for biodiversity experiencing extreme habitat loss and declining species abundance due to deforestation and other anthropogenic activity (Myers et al., 2000). Field work was conducted at Reserva FCAT (Fundación para la Conservación de los Andes Tropicales; 00° 23' 28" N, 79° 41' 05" W), a 603-ha private reserve within the Mache-Chindul Ecological Reserve in Esmeraldas Province. The average temperature in the area is between 23-25°C and annual precipitation is approximately 2,500-3,000 mm, with the wet-season occurring from late December to June (Clark et al., 2006). At our site, monocultures of economically valuable crops (e.g., cacao, African oil palm) and deforested pasture created for cattle are prevalent and increasing. As in much of the neotropics, these alterations to the landscape have created prominent fragmentation in the rainforests of northwestern Ecuador, disrupting connectivity and normal species interactions (Van Der Hoek, 2017).

Study System

Melastomataceae is a highly diverse pantropical plant family (nearly 5,000 described species), with the majority of species located in the neotropics (Renner et al., 2001). Melastomes have positively photoblastic germination requirements, thus many species of neotropical Melastomes are gap-dependent and can act as pioneers in regenerating habitat (Putz, 1983; Ellison et al., 1993; Pearson et al., 2002). *Miconia* species are generally an important group of Melastomes in the neotropics because they produce a large amount of small, fleshy fruits year-round, which provides a reliable food source for frugivores, especially birds, when fruit is scarce during the dry season (Silveira et al., 2013; Messeder et al., 2022). The species of *Miconia* shrub used in this study could not be identified to the species level but constitutes a single morphospecies. The species produces fruits that are small, round, watery, and white when ripe. Each fruit contains an estimated 50-150 tiny (< 1mm), tan seeds suspended in watery pulp near the center of the fruit (personal observation).

White-bearded Manakins (*Manacus manacus*) are particularly tolerant of anthropogenic disturbance and are commonly found along forest edges near pasture and secondary forest. These sites are also abundant in gap-dependent pioneer plant species, including various Melastomataceae. Manakins at our field site have been observed eating whole fruits from the species of *Miconia* shrubs used in this study. This is especially true of *M. manacus*, which tend to situate their lek sites in disturbed secondary forest and along forest edges where these gap-dependent *Miconia* shrubs are also abundant. *M. manacus* are the most common manakin species at our field site, and thus were used for the majority of gut passage trials in this study.

In this study, the following manakin species were used at least once in gut passage trials: *Manacus manacus*, *Lepidothrix coronata* (recently proposed to be renamed *Lepidothrix velutina*; Moncrieff et al., 2022), *Ceratopipra mentalis*, and *Cryptopipo holochlora*. These species are sexually dimorphic, lekking species, except for *C. holochlora*. Adult females of dimorphic manakin species in the area are not easily distinguishable from juveniles of either sex, as females maintain their green plumage present in juveniles into adulthood. Therefore, dimorphic manakin species in this study were categorized as either “adult male” or “green-plumaged” rather than male/female.

Gut passage trials

Gut passage trials were performed over the course of eight weeks from early June to late July, 2022 on wild birds. Each individual gut passage trial began with the capture of a manakin in a ground-level mist net. Mist netting to capture manakins for gut passage trials took place in regenerating forest surrounding known lek sites of *M. manacus*. Following removal from the mist net, each bird experienced a 45-minute fasting period to ensure that the majority of previously consumed seeds had left the bird’s digestive tract

(Levey, 1986; Worthington, 1989). During this period, birds were also banded and processed before being returned to a fabric mist-netting bag for the remainder of the fasting period. All manakins captured during the study period, regardless of sex or age class, were used in gut passage trials, except for recaptured manakins that had already undergone a trial, or birds that showed any indication of injury or illness.

Following the fasting period, birds were fed 1-4 whole *Miconia* sp. fruits that had been previously collected from distinct *Miconia* plants. Plants were marked to avoid resampling, and thus each gut passage trial used fruits a different *Miconia* shrub. The number of fruits fed to a given bird varied depending on the individual's cooperation and condition. Ripe fruits (as determined by visual inspection) used in these experiments were collected < 24 hr prior to use in gut passage trials and stored in dry and dark conditions prior to use in the experiment. Immediately after feeding, birds were placed into a small, covered pet carrier with a perching spot for 45-60 minutes, then released. Fecal samples containing seeds were collected from paper placed at the bottom of the carrier and processed for the experimental germination procedure the same day (below).

Germination experiment

We compared percent germination of gut-passed seeds and control seeds from non-digested fruits using a paired experimental design. For a given experimental trial, gut-passed and control seeds were sourced from the same plant. To enable extraction from feces (gut-passed treatment) or fruits (non-digested treatment) while minimizing handling effects, seeds were suspended in a small amount of sterile water and removed gently with a sterile metal spatula. Extracted seeds were then placed on sterile germination paper inside plastic petri dishes. For each paired trial, the same number of seeds were placed in control and gut-passed petri dishes. However, due to variation in the number of seeds that were recovered following gut passage, the number of seeds obtained varied among trials (mean: 31.83 ± 1.72 SEM, range = 6-42). Petri dish pairs were placed adjacent to one another near a window in the lab at the field station to allow exposure to indirect natural sunlight on a natural light/dark cycle. Germination paper was kept damp as needed using a spray bottle filled with sterile water. Control seeds received the same amount of water as paired gut-passed seeds and were watered at the same times. All petri dishes were monitored together at least 1-2 times per week to record the number of germinated seeds.

Statistical Analysis

To compare the effect of gut passage on the distribution of germination times across all seeds, we fit a linear mixed-effects model using the “nlme” package in R (Pinheiro et al., 2017); the model included time elapsed until germination as a response variable, treatment (i.e., gut-passed vs. non-digested) as a predictor variable, and dish nested within trial as a random effect. This hierarchical random effect structure (i.e., dish within trial) accounted for the fact that seeds belonging to the same trial (i.e., paired gut-passed and non-digested seeds) were derived from the same *Miconia* plant, and seeds in the same dish (in the case of the gut-passed treatment) passed through the same manakin. To test whether gut passage influences the variance of germination times, we calculated the within-dish variance in germination time for all gut-passed and non-digested seeds in each trial. We then conducted a paired t-test to compare these variances in germination times between treatments.

To examine germination success at various timepoints, we conducted two-sided, paired t-tests comparing the percent of gut-passed versus non-digested seeds that had germinated at 20, 40, 60, and 80 days after planting. Because seeds were not checked for germination on a daily basis, the percent germination recorded closest to the 20-day interval desired was used for each 20-day interval to compare mean germination success at each of these points (20-day timepoint mean: 20.17 days, SD: 1.14 ; 40-day timepoint mean: 40.32 days, SD: 1.60; 60-day timepoint mean: 60.80 days, SD: 2.01; 80-day timepoint mean: 80.20 days, SD: 2.69). We conducted paired t-tests to compare mean germination success of gut-passed seeds relative to paired controls for each of the following groups, which are comparisons that were allowed by our sample sizes: all manakin species together, *L. coronata* only, *M. manacus* only, green-plumaged *M. manacus* only, and adult male *M. manacus* only. Statistical tests and figures were conducted

and generated in RStudio Version 1.4.1106 (RStudio Team, 2020) and JMP Version 17.00 (JMP, 2021). All descriptive statistics are presented as means \pm 1 SEM.

RESULTS

We conducted a total of 42 gut passage trials on four manakin species: *M. manacus* (n = 30; 6 adult males, 24 green-plumaged), *Lepidothrix coronata* (n = 9; all green-plumaged), *Ceratopipra mentalis* (n = 2; both males), and *Cryptopipo holochlora* (n=1; sex unknown).

The mean germination time of all seeds combined that were gut-passed by manakins (GP) was 46.73 days (\pm 0.58 SE), while seeds from the non-digested treatment (ND) had a mean germination time of 51.83 days (\pm 0.63 SE). Based on the linear mixed-effects model, the effect of gut passage on germination time was significant ($t = 3.25$, $df = 35$, $p = 0.003$), with gut-passed seeds germinating significantly earlier than non-digested seeds (Fig. 1). The variance in germination times (i.e., the degree to which seeds within a treatment varied in time elapsed prior to germination) did not significantly differ between gut-passed and non-digested treatments (paired t-test: $t = 1.12$, $df = 30$, $p = 0.27$; Fig. 2).

When all manakin species were considered together (n = 43), gut-passed seeds had significantly higher germination success than non-digested seeds at 20 days ($t = 2.16$, $df = 40$, $p = 0.01$) and 40 days ($t = 2.66$, $df = 40$, $p = 0.01$), and still higher but not statistically different at 60 days ($t = 1.76$, $df = 40$, $p = 0.09$) or 80 days ($t = 1.67$, $df = 40$, $p = 0.10$; Fig. 3A).

When considering only the gut passage trials performed with *L. coronata* (n = 9), germination success of *Miconia* was significantly higher at all timepoints examined (20 days: $t = 2.07$, $df = 8$, $p = 0.04$; 40 days: $t = 2.88$, $df = 8$, $p < 0.006$; 60 days: $t = 2.072$, $df = 8$, $p = 0.03$), except for the 80-day timepoint, which was marginally significant ($t = 1.45$, $df = 8$, $p = 0.07$; Fig. 3B).

Germination success of seeds passed by *M. manacus* (n = 30) were significant at 20 days post-planting (20 days: $t = 1.68$, $df = 29$, $p < 0.0499$, but not at any other post-planting timepoints (40 days: $t = 0.02$, $df = 29$, $p = 0.51$; 60 days: $t = 0.28$, $df = 29$, $p = 0.39$; 80 days: $t = 0.22$, $df = 29$, $p = 0.59$; Fig. 3C). For green-plumaged *M. manacus* individuals (n = 24), we found that gut-passed seeds had marginally significantly higher mean germination success than non-digested seeds at 20 days ($t = -1.587$, $df = 32.94$, $p = 0.06$) and insignificant differences in germination success between treatments at all other timepoints (40 days: $t = -0.18$, $df = 42.64$, $p = 0.42$; 60 days: $t = 0.09$, $df = 43.96$, $p = 0.54$; 80 days: $t = -0.00$, $df = 32.39$, $p = 0.50$; Fig. 3D). Results were similar for differences between treatments at germination timepoints for adult male *M. manacus* (n = 6; 20 days: $t = -1.00$, $df = 5$, $p = 0.18$; 40 days: $t = -1.64$, $df = 6.15$, $p = 0.08$; 60 days: $t = -0.40$, $df = 9.94$, $p = 0.35$; 80 days: $t = 0.42$, $df = 6.28$, $p = 0.65$; Fig. 3E).

DISCUSSION

Here, we tested the effects of gut passage by frugivorous birds on the germination of a neotropical melastome. Overall, our results supported the hypothesis that gut passage by manakins accelerates the germination of seeds from this species of *Miconia* shrub. Because our experimental design involved removing the pulp from non-digested seeds and the feces surrounding gut-passed seeds, the gut-passage effects observed in this experiment are expected to be primarily attributable to scarification rather than de-inhibition or fertilization. Surprisingly, we found a significant difference among manakin species in the magnitude of gut passage effects, with seeds passed by *coronata* exhibiting more accelerated germination relative to controls, than seeds passed by *M. manacus*. However, there was no significant difference in mean germination success at any time points between non-digested and gut-passed seed treatments by green-plumaged, nor adult male *M. manacus*, suggesting that gut-passage effects are not dependent on manakin age or sex. Overall, these results provide evidence that seed scarification by manakin gut passage may facilitate earlier germination in a gap-dependent *Miconia* shrub, which may provide this species, and other pioneer melastomes, a competitive advantage to establish in secondary forest habitats and facilitate rainforest regeneration.

Our results are consistent with past melastome germination studies that found gut passage has more of an effect on seed germination times than percent germination, though the direction of these effects have differed depending on the species of melastome and frugivore involved (Ellison et al., 1993; Traveset & Verdú, 2002; Silveira et al., 2012). In our study, gut-passed seeds had significantly higher germination success at earlier timepoints (i.e., 20 and 40 days post-planting), but not at the final timepoint (80 days post-planting). However, the distribution of germination times between treatments revealed that most gut-passed seeds germinated significantly earlier than non-digested seeds. While earlier germination is not always beneficial, and can even be fatal to seeds in conditions where dormancy may be favorable (e.g. pathogen attack, water limitations, and unfavorable light conditions), in the hypercompetitive setting of a regenerating site, seeds of pioneer species that germinate more rapidly may have an advantage over those that take longer to germinate (Janzen, 1981; Murray, 1988; Kelly, 1998; Robertson, 2005; Verdú & Traveset, 2005).

Variance in time elapsed until seed germination has also been shown to be differentially affected by gut passage, with some studies finding that gut passage increases heterogeneity in emergence times by altering seed dormancy and others finding that it increases evenness in germination times (Izhaki & Safriel, 1990; Traveset & Verdú, 2001). It has been suggested that greater variation in germination time should be favored evolutionarily to avoid synchronous emergence that could be detrimental to seed fate, though whether this strategy is beneficial to the plant involved likely depends on the system (Murray 1988; Izhaki & Safriel, 1990; Traveset & Verdú, 2005). In our study, gut passage had no significant effect on the variance of germination times. However, in the case of gap-dependent pioneers like *Miconia*, consistently early germination could be a preferable strategy in regenerating habitats than a strategy of variable or delayed germination.

Because seeds in our experiment were isolated from fecal matter or fruit pulp, we interpret our results to be due primarily to scarification effects of gut passage. While we did not attempt to directly observe or quantify scarification, other studies that have examined seed coats via electron microscopy after avian gut passage have shown an apparent change in texture and increased porosity in comparison to seeds that had not passed through the gut (Barnea, 1990). However, Traveset's (1998) review, which included 183 plant species in 68 families, showed that scarification effects were small and inconsistent, with only 36% of studies finding positive effects on germination. Since manakins are gulpers (Levey et al., 1987), have rapid gut-passage times (Worthington, 1982; Levey, 1986), and lack a crop and grinding gizzard (Worthington, 1982; Ellison et al., 1993), scarification of the seedcoat during gut passage is likely to be relatively minor (Moermond & Denslow, 1985). The combination of morphological traits that characterize manakin digestion might be ideal for the tiny seeds typical of most melastomes, which may only require minimal scarification to elicit germination. Indeed, seed size has been shown to be an important factor in how gut passage influences germination and has been connected to coevolutionary relationships between fruiting plants and frugivores (Levey, 1987; Howe, 1993; Stiles & Rosselli, 1993; Jordano, 2000; Silveira, 2012).

Although this study focused specifically on the gut-passage effects of manakin frugivory, manakin-mediated dispersal may also benefit melastomes from a spatial perspective. Home ranges of some manakins (e.g., *M. manacus*) are located in disturbed secondary forest, often near forest edges or along pastures. Therefore, manakin movements likely increase the probability that positively photoblastic *Miconia* seeds will be defecated in areas that meet gap-dependent germination requirements (i.e., disturbed areas with increased light). Thus, despite our results showing that gut passage by *M. manacus* had no impact on the germination rate or success of this *Miconia* species, they may still be effective seed dispersal agents in secondary tropical habitats due to their spatial ecology and depulping services. On the other hand, while *L. coronata* significantly accelerated the germination of these *Miconia* seeds, they may be less preferable spatial dispersers for photoblastic melastomes, given that their home ranges tend to primarily encompass less disturbed forest, further from gaps and deforested sites (Skutch, 1969). If Janzen-Connell effects are important in this system, it is important to note that female manakins – which generally have larger home ranges than adult males (e.g., Tori et al., 2016), which spend most of their time in lek areas (Snow, 1962; Kridjer et al., 1997) – may provide more favorable seed dispersal services. The larger home ranges and

potentially greater movements by female manakins (Théry, 1992; Karubian & Durães, 2009) between fragmented habitats could have positive implications for seed dispersal and regenerating land via their delivery of gut-passed, pioneer species seeds into these habitats.

Future studies with larger sample sizes could investigate the metabolic and gut-retention time differences between manakin species, sexes, and ages in relation to germination success of gut-passed seeds. We did not record gut-retention times in this study because holding cages during gut-passage trials were covered to minimize stress; however, our specimens seemed to have protracted gut passage times, perhaps due to the stress of capture and feeding, that were inconsistent with previously published gut-retention times of captive manakins. Additionally, despite attempting to primarily investigate gut-passage effects of scarification in this study by controlling for the effects of fertilization and depulping, it is possible that less well-understood gut passage effects (e.g., microbiome interactions in the digestive tract) may have had an influence on germination results. More research determining the specific mechanisms by which scarification of the seed by gut passage alters germination of pioneer species (i.e., how scarification affects light perception and germination times) would also be helpful in the context of restoration ecology, especially in heavily fragmented neotropical forests. Field studies comparing the survival and growth between gut-passed and non-digested seeds could also be conducted in the future to better understand how the effects on germination time observed in this study may influence pathogen avoidance or ultimate recruitment success under natural conditions.

The findings of our study suggest that manakins offer high-quality seed dispersal services to a gap-dependent *Miconia* shrub in northwest Ecuador via their positive gut-passage effects that result from minor scarification of the seed coat, which accelerates germination of defecated seeds. *Lepidothrix coronata* in particular provides this *Miconia* shrub with the advantage of earlier germination, which may enhance seedling establishment in regenerating habitats. Considering that year-round *Miconia* fruit production provides an important food source to a wide variety of animals during the dry season when fruit is less abundant (Messeder et al., 2022), working toward understanding the innerworkings of *Miconia* dispersal by its most beneficial dispersers is valuable, especially in the context of rainforest restoration. Variations of the manakin-melastome relationship exemplified in this study are widespread throughout the neotropics, thus it is worth further investigating the role this plant-frugivore mutualism takes in shaping the succession of increasingly threatened tropical rainforest.

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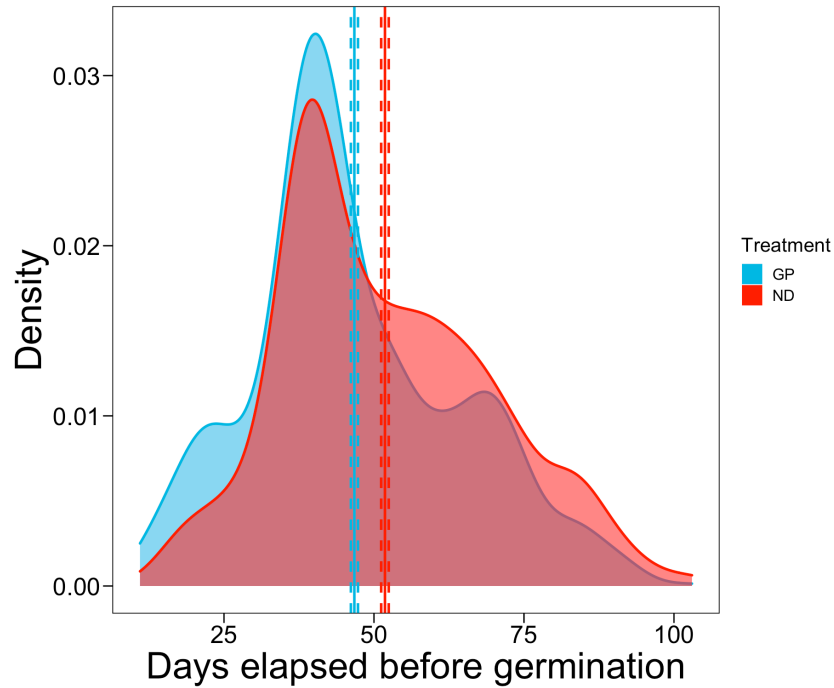
RESULTS CONTINUED (FIGURES)

Figure 1. Distribution of germination times of all seeds between seed treatments

Gut-passed seed treatment (GP) germination times are skewed significantly to the left relative to those of non-digested, control treatment seeds (ND). Solid lines represent means of GP and ND treatments, while dashed lines represent ± 1 SEM (GP = 46.73 days \pm 0.58 SEM ; ND = 51.83 days \pm 0.63 SEM).

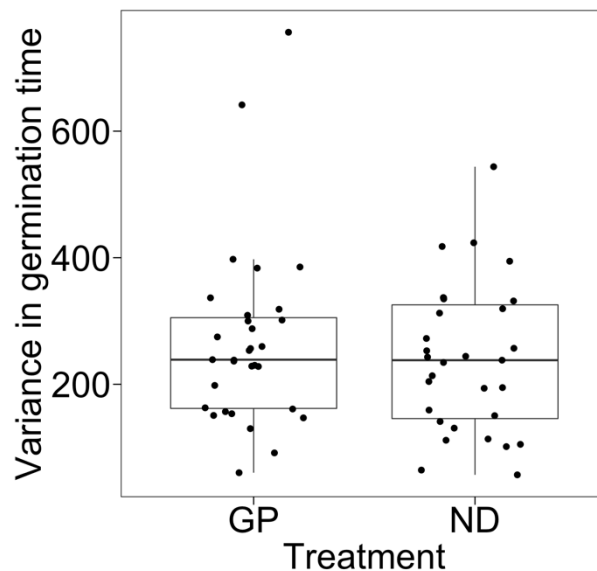


Figure 2. Variances of germination times between seed treatments

There was no significant difference in variance in germination time between gut-passed (GP) and non-digested, control (ND) seed treatments (paired t-test: $t = 1.12$, $df = 30$, $p = 0.27$).

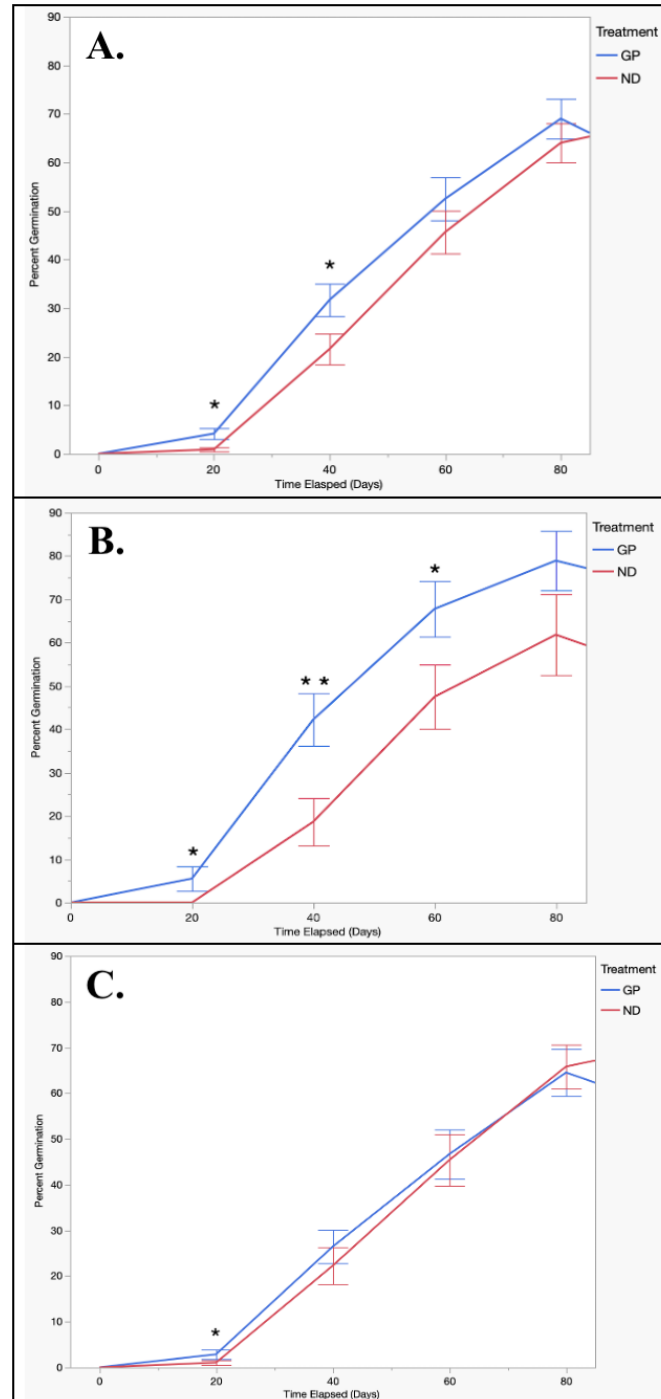
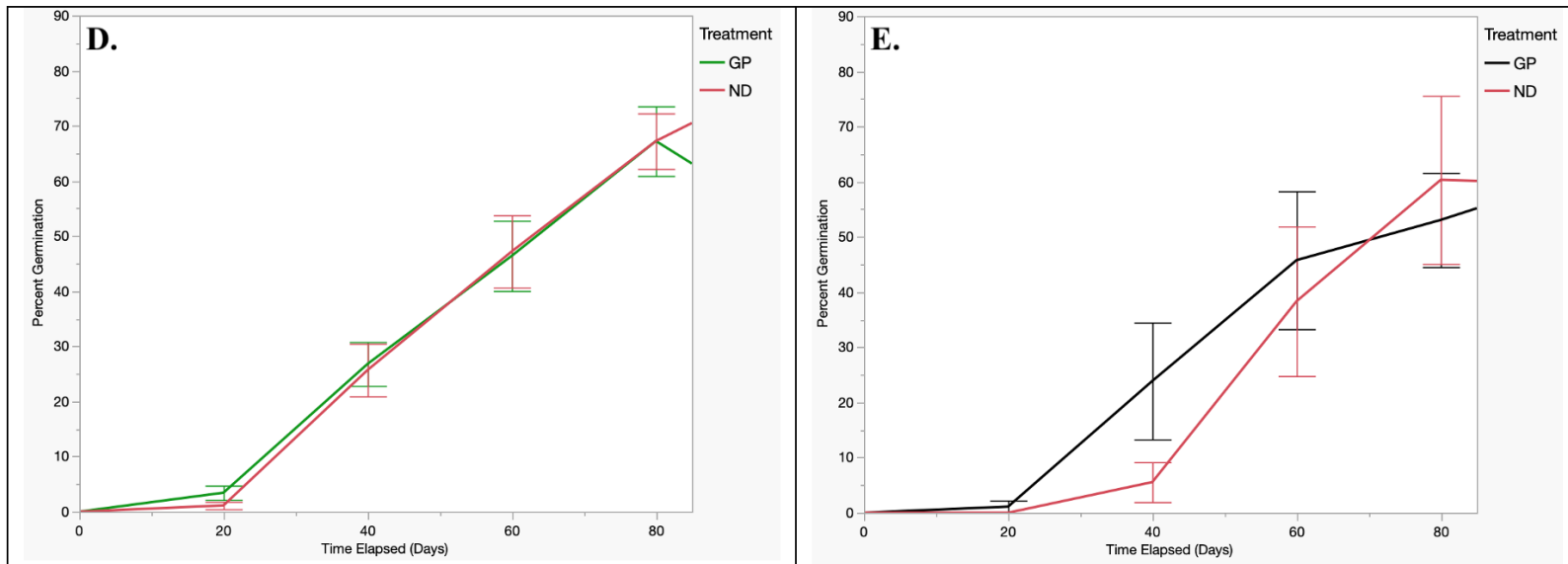


Figure 3. Timepoint-specific germination success by manakin species

Mean percent germination of seeds gut-passed by manakins (GP) vs. non-digested control seeds (ND) at 20-day intervals after planting, when considering trials involving: **A.** All manakin species combined; **B.** *Lepidothrix coronata*; **C.** *Manacus manacus*. Significance of $p < 0.05$ is represented by “*” and “**” for significance of $p < 0.01$. Error bars represent ± 1 SEM.

**Figure. 3 (continued)**

D. Green-plumaged (female or juvenile) *M. manacus*. **E.** Adult male *M. manacus* (ND). No significance ($p > 0.05$) was detected at any timepoints. Error bars represent ± 1 SEM.

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