

# Avian fruit ingestion differentially facilitates seed germination of four fleshy-fruited plant species of an Afrotropical forest

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**Background and aims** – The effects of gut treatment on the germination of animal-dispersed seeds are critical for the recruitment of many fleshy-fruited plant species, and hence for forest dynamics. However, these effects remain poorly studied especially in African plant species. In this paper, we aim to investigate the effects of gut treatment on the germination capacity and mean germination time of four common plant species of an Afrotropical cloud forest.

**Methods** – We fed *Xymalos monospora* fruits to its three main avian dispersers to investigate different responses among frugivore species. Next, fruits of four plant species were fed to the most dominant avian disperser, *Andropadus milanjensis*, to compare responses among plant species. Germination capacity and mean germination time were compared among gut-passed seeds, manually depulped seeds and intact fruits.

**Key results** – Germination of *Xymalos monospora* seeds was up to nine times more likely and almost twice as fast after bird ingestion compared to intact fruits. An increase in germination capacity, but not in mean germination time, was also detected in four sympatric plant species after ingestion by *Andropadus milanjensis*. The positive effects of seed ingestion on germination was mainly by fruit pulp removal; only one plant species showed an indication of the combined effect of fruit pulp removal and seed coat scarification on its germination success.

**Conclusion** – Our results indicate that fruit ingestion of four common African forest plant species positively affected germination capacity and mean germination time, and that recruitment success of these plant species may therefore largely depend on their seed dispersers.

**Key words** – Africa, bird, frugivory, gut passage, seed dispersal, *Xymalos monospora*, *Turraea holstii*, *Psychotria lauracea*, *Leptonychia usambarensis*.

## INTRODUCTION

Given the large number of tropical plant species that rely on frugivorous animals for seed dispersal (Howe & Smallwood 1982) and their various effects on seed germination and recruitment (Schupp 1993), frugivorous vertebrates may affect plant distribution and diversity both in short and long term (Wright 2010). Knowledge on how frugivores affect plant recruitment is therefore crucial to understand how they shape forest dynamics.

Ingestion of seeds by vertebrate dispersers may either positively or negatively affect seed germination, or have no apparent effect. Germination facilitation or inhibition may be affected by ingestion in at least three different ways: (i) by the scarification of the seed coat increasing their permeability for water and gases (scarification effect); (ii) by the removal of germination inhibitors and release of osmotic pres-

sure through pulp removal (de-inhibition effect); and/or (iii) by the enhancement of seed germination or seedling growth through the deposition of faecal material around the seed (fertilization effect; mainly for mammal-dispersed seeds) (reviewed by Traveset 1998, Traveset & Verdú 2002). Although both germination capacity and time may be affected, the latter process is seldom studied - especially so in African forest plants.

We here test how avian seed ingestion of four predominant, fleshy-fruited plant species of Afromontane forest affects their germination capacity and mean time to germination. Three frugivores (*Andropadus milanjensis*, *Turdus helleri* and *Tauraco hartlaubi*) jointly disperse up to 80% of the seeds of these plant species in the Taita Hills biodiversity hotspot, south-east Kenya, where the study was conducted (Lehouck et al. 2009b and V. Lehouck, Ghent University, unpubl. res.). We first fed fruits of the common, forest-interior

**Table 1 – Germination of four plant species after gut treatment by *Andropadus milanjensis*.**Gut passage time, percentage of germinated seeds and time to germination of four different fruit species fed to *A. milanjensis*.<sup>1</sup>: Ntrials/Ntrees = number of experimental trials (equals number of bird individuals fed) / number of plant individuals from which seeds were used in the experiment. The sample size is lower compared to that for GPT (see text) because not all seeds were used in germination trials; NA: not available.\*\*\*  $p < 0.001$  \*\*  $p < 0.01$ , \*  $p < 0.05$ . †:  $0.1 < p < 0.05$ , NS:  $p > 0.1$ 

Plant species	<i>Turraea holstii</i>	<i>Xymalos monospora</i>	<i>Psychotria lauracea</i>	<i>Leptonychia usambarensis</i>
Family	Meliaceae	Monimiaceae	Rubiaceae	Sterculiaceae
Fruit type	Capsule, usually 5 seeds	Berry, 1 seed	Drupe, usually 2 seeds	Capsule, 1–5 seeds
Disperser unit and color (n = # fed per experimental trial)	Seed, black and almost completely covered by orange-red aril (n = 5)	Fruit, yellow- orange-reddish (n = 3)	Fruit, red/scarlet (n = 3)	Seed, black with orange-red aril (n = 3)
Mean size of disperser unit (length (mm) × width (mm))	4.5 × 2.5	11.8 × 9.4	4.5 × 5.5	11.1 × 6.9
Ntrials/Ntrees <sup>1</sup>	9 / 1	66 / 21	10 / 5	22 / 7
Median GPT (25–75 percentile) (min)	55 (17–129)	37 (15–115)	51 (29–81)	49 (28–90)
Percentage seeds germinated				
Mean % ± SE				
gut passage	78.1 ± 7.9	68.2 ± 4.5	51.1 ± 10.9	50.0 ± 20.5
control fruit	52.1 ± 17.6	7.4 ± 2.4	27.8 ± 5.9	NA
control seed	74.7 ± 10.3	67.4 ± 4.5	19.8 ± 6.3	50.0 ± 11.5
Wilcoxon signed rank test				
gut-fruit	S = 6.5 NS	S = 297.5 ***	S = 13.5 †	NA
gut-seed	S = -2.0 NS	S = 10.0 NS	S = 19.0 *	S = 0 NS
fruit-seed	S = -12.0 *	S = 343.5 ***	S = 5.0 NS	NA
Time to germination				
Mean days ± SE				
gut passage	132 ± 10	64 ± 5	139 ± 14	145 ± 15
control fruit	117 ± 23	119 ± 14	137 ± 14	NA
control seed	137 ± 9	56 ± 4	154 ± 5	134 ± 14
Wilcoxon signed rank test				
gut-fruit	S = 1.0 NS	S = -6.0 NS	S = -3.5 NS	NA
gut-seed	S = -2.0 NS	S = 273.0 **	S = -4.0 NS	S = 2.0 NS
fruit-seed	S = -1.5 NS	S = 9.0 NS	S = -0.5 NS	NA

tree *Xymalos monospora* to these three avian dispersers to investigate different germination responses among frugivore species. Next, fruits of four predominant plant species (*Xymalos monospora*, *Turraea holstii*, *Psychotria lauracea* and *Leptonychia usambarensis*) were fed to the most dominant avian disperser, *Andropadus milanjensis*, to compare responses among plant species. Comparison of germination capacity and mean germination time of gut-passed seeds, manually depulped seeds and intact fruits allows us to reveal the underlying mechanisms of avian gut treatment effects on seed germination (Samuels & Levey 2005).

## METHODS

Fieldwork was conducted in the heavily fragmented Taita Hills forest, a biodiversity hotspot in south-east Kenya (03°20'S 38°15'E, alt. 1200–2208 m, rainfall 600–2300 mm/yr) from August 2004 to August 2006. The regional climate is dominated by heavy rains during the cold season (March–May/June) and short rains during the warm season (October–December), with fruit densities usually peaking from August to January.

Birds were captured with mist-nets and individually held in clean cotton bags to minimize stress during gut passage

**Table 2 – Germination of *Xymalos monospora* seeds after gut treatment by its three main avian frugivores.**

Percentage of and time to germination of *X. monospora* seeds after gut passage (*gut*; three frugivorous bird species) and two control treatments (intact *fruit* and manually depulped *seed*). Between-treatment differences were tested pairwise for statistical significance using Wilcoxon signed rank tests (see text for details). <sup>2</sup>: N = number of experimental trials (equals number of bird individuals fed, with each bird fed 3 fruits). The sample size is lower compared to that for GPT (see text) because not all seeds were used in germination trials.

\*\*\* p < 0.001 \*\* p < 0.01, \* p < 0.05. †: 0.1 < p < 0.05, NS: p > 0.

Bird species	<i>Andropadus milanjensis</i>	<i>Turdus helleri</i>	<i>Tauraco hartlaubi</i>
Family	Pycnonotidae	Turdidae	Musophagidae
Mean body weight (range) (g)	43 (15–55)	70 (53–85)	230 (195–275)
Median GPT (25–75 percentile) (min)	33 (24–54)	34 (26–57)	40 (26–57)
N <sup>2</sup>	66	21	31
Percentage seeds germinated			
Mean % ± SE			
gut passage	68.2 ± 4.5	46.0 ± 9.0	33.1 ± 4.5
control fruit	7.4 ± 2.4	13.6 ± 10.2	18.7 ± 11.6
control seed	67.4 ± 4.5	56.3 ± 8.9	41.8 ± 3.6
Wilcoxon signed rank test			
gut-fruit	S = 297.5 ***	S = 20 *	S = 51.5 *
gut-seed	S = 10 NS	S = -12.5 NS	S = -37 NS
fruit-seed	S = 343.5 ***	S = -16.5 †	S = -57.5 **
Time - to - germination			
Mean days ± SE			
gut passage	64 ± 5	94 ± 15	84 ± 4
control fruit	119 ± 14	160 ± 1	118 ± 4
control seed	56 ± 4	79 ± 14	79 ± 2
Wilcoxon signed rank test			
gut-fruit	S = -6 NS	S = -0.5 NS	S = -67 ***
gut-seed	S = 273 **	S = 3.5 NS	S = 17 NS
fruit-seed	S = 9 NS	S = 1.5 NS	S = 94 ***

time (GPT) experiments. All individuals were fed with bananas to clear their guts before feeding them three to five disperser units (i.e. seeds embedded within fruit pulp or accompanied by their aril) depending on the plant species (table 1; see Lehouck et al. 2009a for more methodological details). Whereas sample sizes of *Xymalos monospora* fruits allowed studying differential effects of fruit ingestion among the three frugivorous bird species, differences in germination response to ingestion of three other plant species could only be studied for *Andropadus milanjensis*. The number of units fed per individual bird mimics feeding bouts observed in the field (Lehouck et al. 2009b). Cotton bags were checked every five minutes until all seeds were defecated (the few regurgitated seeds were omitted from the dataset). All birds were in good condition when released (mostly within one hour and not later than three hours after being caught; see Lehouck et al. 2009a for details).

Individual time intervals between fruit swallowing and

seed defecation were recorded as a measure of GPT. After retrieval, seeds were individually planted at c. 0.5 cm depth in a 50:50 mixture of sand and fertile soil (sieved, collected from a single forest site) maintained outdoors in large, rectangular trays topped with a lid of transparent plastic to retain moisture and deter possible seed predators. All treatments were paired, i.e. for each retrieved seed, one manually depulped seed (i.e. with fruit pulp or aril removed manually) and one intact fruit were collected from the same tree (see table 1 for the number of plant individuals used per species) and planted on the same day. For logistic reasons, up to three days elapsed between feeding and planting of the seeds. However, storage duration had no significant effect on germination probability (Proc Genmod in SAS; storage duration:  $\chi^2_2 = 1.61$ , df = 2, p = 0.45; storage duration\*treatment effect:  $\chi^2_1 = 0.15$ , p = 0.70). We introduce the term ‘experimental trial’ to indicate the total of the retrieved seeds per individual bird (equal to the number of fruits fed, i.e. 3–5) and both controls (i.e.

manually depulped seed and intact fruit). This setup enables us to separately test effects of avian seed ingestion on germination through fruit pulp removal (by comparison of manually depulped seeds with intact fruits) or through scarification of the seed coat (by comparison of manually depulped seeds with gut-passed seeds), as well as their combined effect (see also Samuels & Levey 2005). After planting, seeds were watered daily and checked every three days for seedling emergence (i.e. when part of a shoot becomes visible above-ground), until three months after the last seed germinated. At the end of the experiment, the soil was carefully sieved to confirm that no intact seeds remained (all seed remains were soft and rotten and therefore not viable).

Measurements of GPT, time to germination and of percentage of germinated seeds were averaged per experimental trial to account for individual variance (Charalambidou et al. 2003) and tested for treatment effects with non-parametric Wilcoxon signed rank tests.

## RESULTS

*Xymalos monospora* seeds did not significantly differ in median GPT values among the three bird species (table 2,  $\chi^2_2 = 0.62$ ,  $p = 0.73$ ) and their germination capacity and mean time to germinate were similarly affected after ingestion by these frugivores. The likelihood of germination of *X. monospora* seeds increased up to nine fold after bird ingestion compared to seeds from intact fruits, while no significant differences were found between ingested and manually depulped seeds. The latter seeds tended to germinate slightly earlier than ingested ones, while germination of seeds from intact fruits took up to twice as long, although differences in germination speed among treatments were not always significant due to a high degree of variability and small sample sizes (table 2).

We found a similar, albeit less strong, non-significant positive effect of seed ingestion by *Andropadus milanjensis* on the germination capacity of *Turraea holstii* compared to seeds surrounded by pulp, while ingestion of *Psychotria lauracea* enhanced the germination capacity both with respect to manually depulped seeds and intact fruits. For *Leptonychia usambarensis*, manually depulped and defaecated seeds germinated equally well; however, no comparison with arillated seeds could be made. Mean time to germination was not significantly affected in any of these three plant species (table 1; no data for ingestion of these plant species by *Turdus helleri* and *Tauraco hartlaubi* available). GPT significantly differed between plant species ( $\chi^2_3 = 9.1$ ,  $p = 0.03$ ), with longer transit times for smaller diaspores (Spearman rank GPT-diaspore length:  $r = -0.24$ ,  $p = 0.003$ ). However, GPT did not significantly affect germination capacity or mean germination time within any of these species (Kruskall-Wallis and Spearman rank correlation, all  $p > 0.05$ ).

## DISCUSSION

The ability of seeds to remain viable after ingestion by frugivores is crucial for the population dynamics of endozoochorous plant species and is believed to have shaped the evolution of plant-frugivore interactions (Murray et al. 1994). Seed ingestion by vertebrate dispersers may have a positive,

neutral or negative effect on seed germination capacity and time (reviewed by Traveset 1998, Traveset & Verdú 2002). In our study, *Xymalos monospora* seeds had a higher probability of germinating, and germinated faster, after ingestion compared to seeds that remained embedded in pulp, as would be the case in absence of dispersal by frugivores. Similarly, positive and/or neutral effects of gut treatment by *Andropadus milanjensis* on germination were observed in three other plant species of which seeds are predominantly dispersed by the same suite of frugivores. Because GPT did not significantly affect germination capacity or mean germination time within any of these plant species, inter-specific differences in germination response of plant species were probably not caused by differences in GPT, but rather by differences in mechanical or physiological effects of seed ingestion on seed coat structure and/or permeability. Since manually depulped seeds germinated equally well as defaecated seeds in most of our experimental trials, positive effects of seed ingestion are mainly through fruit pulp removal, probably because of the removal of germination inhibitors therein and release of osmotic pressure (deinhibition effect; Robertson et al. 2006). Additional effects of seed coat scarification were evident in at least one plant species (*Psychotria lauracea*), as gut-passed seeds in this species germinated better than manually depulped seeds. While the fitness advantage of enhanced germination capacity is clear, advancement of seed germination in time may be unfavourable, rather than favourable, under unpredictable environmental conditions when most species built up a soil seed bank, i.e. an assemblage of viable seeds in the soil from which germination may be spread over time through dormancy (Fenner 1992). However, plant species with non-dormant, thin-coated seeds such as *Xymalos monospora* are generally believed to benefit from advanced germination as it reduces the exposure time of the sensitive seeds to predators and microbial or fungal attack (Schupp 1993, Vázquez-Yanes & Orozco-Segovia 1993, Traveset & Verdú 2002). Once germinated, seedlings may then continue to grow fast or remain 'dormant' in a seedling bank until conditions are favourable, a mechanism that appears to be common in tropical regions (Vázquez-Yanes & Orozco-Segovia 1993, Lehouck et al. 2009c).

Although verification under field conditions (natural setting and different burial depths) is needed to confirm these findings (Rodríguez-Pérez et al. 2005, Robertson et al. 2006), our results suggest that avian seed ingestion provides both direct and indirect positive effects on recruitment through effects on germination capacity and time, respectively, to a degree that varies among plant species. In forests where a large number of plants are dispersed by a limited number of avian frugivores, as is the case in the Taita Hills, loss of these frugivores might have far reaching consequences for the recruitment of fleshy fruit producing plants (see also Lehouck et al. 2009b) and forest dynamics in general. Global changes, such as habitat loss and fragmentation or climate changes, may therefore not only pose direct threats to forest persistence, but also indirectly influence forests through effects on their mutualistic counterparts, in this case seed dispersers.

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