

Effects of dung presence, dung amount and secondary dispersal by dung beetles on the fate of *Micropholis guyanensis* (Sapotaceae) seeds in Central Amazonia

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ABSTRACT. The study of seed fate is crucial for understanding fruit–frugivore interactions. One factor that can greatly influence the fate of seeds dispersed through mammal defecation, is the dung that accompanies the seeds. Dung attracts dung beetles and rodents; the former eat and bury dung, the latter feed on seeds. In this study the fate of *Micropholis guyanensis* subsp. *guyanensis* seeds surrounded by no dung and by 5, 10 and 25 g of howler monkey dung was followed until seedling establishment. The depths at which dung beetles bury the seeds were measured, and the effect of burial depth on seedling emergence was determined. Although initial seed fates differed among treatments, the same percentage of seedlings established from seeds without faecal material, and from seeds with 5, 10 and 25 g of dung. However, a significantly higher proportion of seedlings established from buried seeds when compared to seeds that remained on the surface. The percentage of seedlings establishing in a controlled germination experiment decreased significantly with increasing burial depth. The effect of dung beetle activity should be taken into consideration when assessing the role that mammal endozoochory plays on seed dispersal ecology of tropical plants. These results provide further evidence for the emerging realization that the study of secondary seed dispersal and post-dispersal events is crucial for a more complete understanding of plant regeneration.

KEY WORDS: dung beetles, *Micropholis*, Sapotaceae, seed dispersal, seed fate

INTRODUCTION

A crucial aspect in understanding fruit–frugivore interactions is the fate of seeds after deposition by primary dispersers and until seedling establishment

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(Herrera *et al.* 1994, Jordano & Herrera 1995, Schupp & Fuentes 1995). Biotic and abiotic factors that affect a seed during this phase are likely to have a large influence on the structure of plant communities (Chambers & MacMahon 1994). Also, factors that affect the fate of seeds dispersed by a frugivore will ultimately determine the degree to which the fitness of a plant depends on the primary seed dispersal agent (Coates-Estrada & Estrada 1988).

One factor that can greatly influence the fate of seeds dispersed through mammal defecation, is the faecal material that accompanies the seed rain. The presence of dung is an intrinsic characteristic of the primary dispersal event and should be considered when assessing the fate of seeds. Few studies have taken into account this aspect of mammalian seed dispersal (Andresen 1999, Chapman 1989, Estrada & Coates-Estrada 1991, Feer 1999, Janzen 1986, Shepherd & Chapman 1998).

Faecal material attracts dung beetles and rodents; the former eat and bury dung, the latter feed on seeds. A few studies have shown that dung beetles can act as secondary seed dispersers by accidentally burying some of the seeds present in dung, and that this behaviour considerably reduces the probability of seed removal by rodents (Andresen 1994, 1999; Estrada & Coates-Estrada 1991, Feer 1999, Shepherd & Chapman 1998, Vulinec 1999). These studies have also shown that the percentage of seeds that are buried by beetles, as well as the depth at which they are buried, are largely a function of seed size and beetle species.

However, the amount of dung surrounding a seed may have a significant effect on the short-term and/or long-term fate of seeds. For example, a larger faecal clump may provide a stronger olfactory cue for both rodents and dung beetles. On the other hand, a seed inside a large faecal clump may have a better chance of avoiding visual detection by rodents than a seed surrounded by less dung (Janzen 1982). Also, seeds in larger faecal clumps are likely to be buried more deeply by dung beetles than seeds in smaller clumps (Andresen 2000).

A few germination experiments with seeds placed at different depths (Feer 1999, Shepherd & Chapman 1998) have indicated that seed burial by dung beetles may not just have a positive effect on seed survival due to diminished detection by seed predators, but that it may also have a negative effect on seedling establishment due to increased emergence failure of seeds buried too deeply. However, it is still necessary to actually follow the fate of seeds dispersed through defecation by mammals and naturally buried by dung beetles until seedling establishment. The fate of such seeds should be compared to the fate of seeds dispersed without dung (e.g. spat out by mammals or regurgitated by birds) and to the fate of seeds dispersed with dung but not buried by dung beetles, in order to accurately assess the overall net effects of the presence of dung and of dung beetle activity on plant regeneration.

The main objectives of this study were to determine the net role of dung

beetle activity on the regeneration of tropical tree *Micropholis guyanensis* subsp. *guyanensis* (A.DC.), and to assess the effects of presence and amount of faecal material on seed fate. To address these objectives I asked the following questions: (1) What are the short- and long-term fates of seeds surrounded by 5, 10 and 25 g of dung, compared to seeds without dung? (2) To which vertical and horizontal distances are seeds with 10 g of dung moved by dung beetles? (3) What is the effect of burial depth on seedling establishment?

STUDY SITE AND SPECIES

Study site

This study was conducted in a 10 000-ha continuous forest reserve (reserve 1501, also known as 'km 41') that is part of the Biological Dynamics of Forest Fragments Project (BDFFP, formerly known as the Minimum Critical Size of Ecosystems Project, Lovejoy & Bierregaard 1990). The study area is located *c.* 90 km north of the city of Manaus (2°30'S, 60°W), in the Brazilian state of Amazonas. Mean annual temperature in Manaus is 26.7 °C and mean annual rainfall is 2186 mm (Lovejoy & Bierregaard 1990), with a drier season between June and October. The area is covered by primary *terra firme* forest (not subject to seasonal flooding), with a 30–37 m tall canopy (Lovejoy & Bierregaard 1990). The flora is very rich in tree species, with the most important families Burseraceae, Sapotaceae, Lecythidaceae and Leguminosae (Rankin-de-Merona *et al.* 1992).

Plant species and primary dispersers

Micropholis guyanensis subsp. *guyanensis* (hereafter called *Micropholis*) is a canopy tree belonging to the Sapotaceae. In the study area Sapotaceae is one of the most abundant families both in terms of number of species (> 70 spp.) and number of trees (Rankin-de-Merona *et al.* 1992). The fruits of *Micropholis* are globose and measure *c.* 25–30 mm in diameter, and contain 1–3 flat and elongate seeds (length: 18 ± 0.3 mm, width: 9 ± 0.2 mm, thickness: 6 ± 0.2 mm; $n = 20$; here and elsewhere means are reported followed by the SE). Mature fruits are produced during the rainy season, in December–January.

Sapotaceae fruits are indehiscent and often have a hard husk. Consequently, many Sapotaceae species are almost exclusively dispersed by arboreal mammals, which have the biting and manipulative abilities to open such fruits (Janson 1983, Julliot 1996, Roosmalen 1985). In the study region Sapotaceae can be very important in the diets of arboreal mammals (Spironello 1999). I found, for example, that howler monkeys (*Alouatta seniculus*) ate and dispersed the seeds of 56 species of Sapotaceae in a 2-y period (Andresen 2000).

In a study of a different subspecies, *Micropholis guyanensis* subsp. *duckeana* (A.DC.), which has slightly smaller fruits (diameter: 20 mm) and seeds (length: 14 mm), Spironello (1999) found that four species of mammals dispersed most of the seeds. The smaller mammal species, kinkajous (*Potos flavus*, Procyonidae)

and capuchin monkeys (*Cebus apella*), dropped over 50% of the seeds under the parent tree, without swallowing them. The two larger mammal species, howler monkeys and spider monkeys (*Ateles paniscus*), swallowed over 80% of the seeds. Other species of arboreal mammals present in the study site that may disperse *Micropholis* seeds through defecation include three additional species of monkeys (*Saguinus midas*, *Pithecia pithecia* and *Chiropotes satanas*, with the latter two acting mostly as seed predators), the coati (*Nasua nasua*, Procyonidae), the tayra (*Eira barbara*, Mustelidae) and the common opossum (*Didelphis marsupialis*, Didelphidae).

The dung beetle community

Most dung beetle species (Scarabaeidae) in the neotropics feed on vertebrate faeces. After finding a dung source, dung beetles quickly relocate a portion of dung, mostly by burying it under the soil, either for feeding or for nest building (Halffter & Edmonds 1982, Hanski & Cambefort 1991a). Beneficial consequences of their behaviour include soil fertilization and aeration (Mittal 1993), an increase in the rate of nutrient cycling (Nealis 1977) and a reduced transmission of some parasites of vertebrates (Bergstrom *et al.* 1976). However, a poorly known ecological role that dung beetles play, and one that may be particularly important in tropical rain forests, is seed dispersal.

There are about 1200 recognized species of dung beetles in the neotropics (Gill 1991) and tropical rain forest areas typically contain 50 to 60 species of dung beetles (Hanski & Cambefort 1991b). In my study area I captured 61 species of dung beetles using pitfall traps baited with fresh howler monkey dung (Andresen 2000).

According to their dung-processing behaviour (see below), 32 species were tunnellers, 10 were rollers, five were dwellers (including *Erysternus* spp.), and 14 were unidentified species (Andresen 2000). Tunneller dung beetles make a burrow close to the dung source and then start provisioning it with dung. On the other hand, roller dung beetles first make a dung ball, which they always move some distance away from the dung source before making a burrow to bury the dung. Finally, dwellers process the dung immediately below the dung pat, or inside it (Cambefort & Hanski 1991, Halffter & Edmonds 1982). Only tunneller and roller dung beetles are likely to move seeds horizontally and/or vertically, and it has been found that tunneller species bury more seeds than roller species of the same size, and are consequently thought to be more active as secondary seed dispersers (Andresen 1999, Estrada & Coates-Estrada 1991, Feer 1999).

Beetle sizes in the study area ranged from 2 to 38 mm, with 30% of the species and 16% of all individuals captured being ≥ 10 mm. Beetle size is directly related to the probability of a seed being secondarily dispersed by dung beetles, and beetles < 10 mm are unlikely to be important in the movement of seeds > 5 mm in length (Andresen 2000, Feer 1999, Vulinec 1999).

METHODS

All experiments were carried out during the rainy season, between January–May 1997, with final checking of seeds buried by dung beetles (see below) in July–August.

Because large amounts of faecal material were needed for conducting the experiments described below, I used howler monkey dung. Howler monkeys are common in the study site, groups are located readily and the collection of large quantities of dung is feasible. Dung was collected in the morning (08h00–10h00), after I located a howler monkey troop when it vocalized at dawn (04h00–06h00). All seeds > 3 mm present in the dung, were removed to avoid interference with experimental seeds. Seeds \leq 3 mm were not found in the dung very often, their removal was not practical, and they were not thought to interfere with experimental seeds. Dung was kept in plastic bags in the shade. Since the freshness of dung is very important for its attractiveness to dung beetles (Halffter & Edmonds 1982, Howden & Nealis 1975), the dung was generally used for experiments on the same day. When the dung was used the next day, it was kept in a cooler box with ice.

Micropholis seeds were either collected from howler monkey defecations or from fruiting trees. The same percentage of seedlings established from seeds of both sources (93% for seeds from defecations ($n = 28$) vs. 96% ($n = 27$) for seeds from trees, $\chi^2 = 0.315$, $df = 1$, $P = 0.57$). In both cases seeds were thoroughly washed until no dung or pulp remained on the seed surface. All seeds were marked with a 50-cm long white nylon thread, which was glued to the seed in a way that did not interfere with germination. The purpose of the thread was to aid in finding the seeds that were moved by dung beetles or rodents.

Seed fate: the effect of presence and amount of dung

Seeds were placed on the forest floor, along transects, one seed every 10 m (distances of even 5 m have been considered to assure independence in seed removal/predation experiments in rainforests; (Blate *et al.* 1998, Burkey 1993)). Each seed was placed next to a 20-cm long wooden stake that had a 5-cm piece of flagging tape at the top. These stakes had been put in place at least 2 d before seeds were set out.

Four treatments were used: seeds without faecal material, and seeds surrounded by 5, 10 and 25 g of dung. The no-dung treatment simulates seeds that have been dispersed in other ways, such as spat out by mammals or regurgitated by birds. The amounts of dung were chosen according to the weights recorded for howler monkey faecal clumps: 48% of all dung in a sample of 13 defecations (4772 g of dung) was in piles that weighed 5–30 g, while 29% was in piles > 30 g and 23% in piles < 5 g. Other researchers have recorded dung-pile sizes within the 5–30 g range for howler monkeys, spider monkeys and coatis (Andresen 1999, Estrada & Coates-Estrada 1991). Only one seed

was used per dung pile, and one dung pile per station, to avoid tangling of the threads during dung beetle activity. It is important to keep in mind, however, that different dung-pile and seed densities may affect the fate of seeds differently (Andresen 2000, Sánchez-Cordero & Martínez-Gallardo 1998, Willson & Whelan 1990).

Treatments were set out simultaneously, alternating them along transects, with a total of 10 seeds/treatment in each transect (i.e. 40 seeds per transect). Seeds from only one source, either from faecal samples or fruits, were used in each transect. Individual transects were separated from each other by at least 50 m and a maximum of three transects were set out on any single day. A total of 16 transects were set out, yielding 160 seeds per treatment. All seeds were set out in the afternoon, between 15h00 and 17h30. This was done to avoid having 'time of day' as a confounding factor since the diurnal and nocturnal dung beetle guilds vary in species composition and biomass (Andresen 2000, Hanski & Cambefort 1991c).

I checked transects the next day, i.e. 18–24 h after the setting-up, and once every 2 wk thereafter, until predation/removal, or seedling establishment (total of 16 wk). For each seed I recorded its fate (seed alive, seed predated/removed, germinating seed, seedling) and location (buried or on the surface). Buried seeds were recognized by the nylon thread emerging from the soil. I only unburied these seeds at the end of the experiment. Seeds placed without dung were never found buried, thus I assumed that seeds with dung that were found buried, had been buried by dung beetles, and not by rodents or other animals. For 95% of the seeds placed with dung ($n = 480$), all the faecal material had disappeared by the time I conducted the first check, and for 96% of seeds that were found buried ($n = 184$), burial was also recorded at this time. Only in the case of eight seeds was burial recorded on the second transect check (after 2 wk). This also argues in favour of burial by dung beetles. Also, most of these seeds were never moved large horizontal distances (see below), which further argues against burial by rodents (P.-M. Forget, *pers. comm.*). Finally, in an experiment conducted with plastic beads instead of seeds, in which all bead burial can consequently be safely assumed to have been done by beetles, beads were buried in exactly the same pattern as were *Micropholis* seeds (Andresen 2000).

Seeds were classified as predated when seed remains were found, and as removed when the seed disappeared. A seedling was considered established when both leaf-like cotyledons were spread out (the leaf-like cotyledons of *Micropholis* are photosynthetic, and seedlings may remain at this developmental stage for many months, before they grow true leaves). When seeds had been moved from the original location, an effort was made to find the seed and/or thread; an area of *c.* 5 m around the location was searched. I never found a seed scatterhoarded by rodents. Thus, seeds that were not found were assumed to have been eaten by rodents. It is important to keep in mind, however, that some of the removed seeds could have been scatterhoarded by rodents (Forget

et al. 1998). Also, some of these seeds could have been removed by dung beetles > 5 m, or buried by dung beetles together with the thread. However, due to the results of experiments in which I used plastic beads (Andresen 2000), I do not think that these two latter events were likely. Ten weeks after the last transect check, the buried seeds that had not emerged as seedlings were unearthed to determine whether the seeds had not germinated or the emerging seedlings had not been able to reach the surface.

Burial depth and horizontal movement of seeds

To determine the vertical and horizontal distances to which dung beetles naturally move seeds, I used marked seeds surrounded by 10 g of dung, following the same experimental setup as for the seed fate experiment, except that I unearthed the seeds after 2 d. I measured the depth of burial of the seeds to the nearest centimetre and horizontal movement to the nearest 5 cm. These seeds were re-used, completing a sample size of 117, but these seeds were never used in the seed-fate experiment.

Effect of burial depth on seedling establishment

To assess the effect of burial depth on seedling emergence, a germination experiment was conducted. Seeds were placed at four depths (0, 1, 5 and 10 cm) in individual plastic cups filled with forest soil. I used 55 seeds for the first treatment and 45 for each of the other three treatments. The plastic cups had drainage holes and were placed on the forest floor, in an area where the understorey and midstorey vegetation had been cleared, but the canopy was intact. A plastic mosquito net covered the cups to prevent seed and seedling predation. The cups received rain-water, but were not artificially watered. The number of seedlings establishing in each treatment was recorded.

RESULTS

Seed fate: the effect of presence and amount of dung

Seventy-three per cent of all seeds used in this experiment ($n = 640$) were predated or removed by seed-eating animals, most likely rodents (although some of the mortality of germinating seeds was also attributable to insects). Seed predation and seed removal were observed 49 and 51% of the times, respectively. Germination of *Micropholis* seeds was observed, on average, after 6 ± 0.2 wk (range: 2–14 wk; $n = 150$), and seedling establishment (as defined above) was observed after 10 ± 0.3 wk (range: 6–20 wk; $n = 73$).

In all the statistical analyses presented below, I pooled data from the 16 transects. I did this after testing for homogeneity of association between the pair of factors being analysed (e.g. amount of dung vs. seedling establishment), among the 16 transects. This test was done by adding 'transect' as a third table factor, and fitting in each case a log-linear model for a three-way frequency table without the 3rd order interaction term (Agresti 1996, Sokal & Rohlf

1995). When such a model fits the observed frequencies ($P > 0.05$) it means that the association between any two factors (e.g. amount of dung and seedling establishment) is the same at any level of the third factor (e.g. transect). For the main tests presented in this section, following the results for the chi-square tests on pooled data, I report the likelihood ratio chi-squared (LR χ^2) statistic for the log-linear model testing for the non-significance of the 3rd order interaction term, in which 'transect' was the third factor.

The overall fate of seeds in the four treatments is shown in Figure 1a. Differences were found in the proportion of seeds predated/removed ($\chi^2 = 68.6$, $df = 3$, $P < 0.01$; partial associations were homogeneous among transects, LR $\chi^2 = 33.2$, $df = 46$, $P = 0.92$) and the proportion of seeds that failed to emerge due to burial depth ($\chi^2 = 86.8$, $df = 3$, $P < 0.01$; partial associations were homogeneous among transects, LR $\chi^2 = 28.3$, $df = 46$, $P = 0.98$). However, these effects offset one another and no differences were observed in the percentage of seedlings establishing in the four treatments ($\chi^2 = 0.92$, $df = 3$, $P = 0.82$; partial associations were again homogeneous among transects, LR $\chi^2 = 48.6$, $df = 48$, $P = 0.44$).

After performing chi-squared post-hoc pairwise comparisons (probabilities adjusted according to Sokal & Rohlf (1995)), I found that seeds in the 0- and 5-g treatments suffered similar predation/removal rates ($P > 0.05$) and that these were significantly higher than predation/removal rates for seeds in the 10- and 25-g treatments (all P s < 0.01). Finally, predation/removal in the latter two treatments was the same ($P > 0.05$).

In terms of the proportion of seeds that failed to emerge, results were in the opposite direction: seeds with no dung, which were never buried by dung beetles, did, as expected, not experience emergence failure. Seeds surrounded by 5 g of dung suffered significantly higher emergence failure than seeds without dung, but significantly lower emergence failure than seeds with 10 and 25 g of dung (all P s < 0.01 in adjusted pairwise comparisons with chi-squared tests). The 10- and 25-g treatments had similar proportions of seeds that failed to emerge ($P > 0.05$).

Higher predation/removal rates in the 0-g treatment were clearly due to these seeds being on the surface. However, for seeds with dung, the amount of dung had a significant effect on the probability of seed burial. Only 16.9% of seeds were buried when surrounded by 5 g of dung, while 47.5 and 48.1% of seeds were buried when surrounded by 10 and 25 g of dung, respectively ($\chi^2 = 43.6$, $df = 2$, $P < 0.01$; partial associations were homogeneous among transects, LR $\chi^2 = 28.8$, $df = 30$, $P = 0.56$). Consequently, in the case of the 5-g treatment, higher predation/removal rates, as well as lower emergence failure, could have been due to less seeds being buried and/or due to lower burial depths for seeds buried by dung beetles. Thus, to test whether the results on seed predation and emergence failure were not simply a consequence of the fact that seeds with 5 g of dung were buried less often than seeds with 10 and 25 g of dung, I

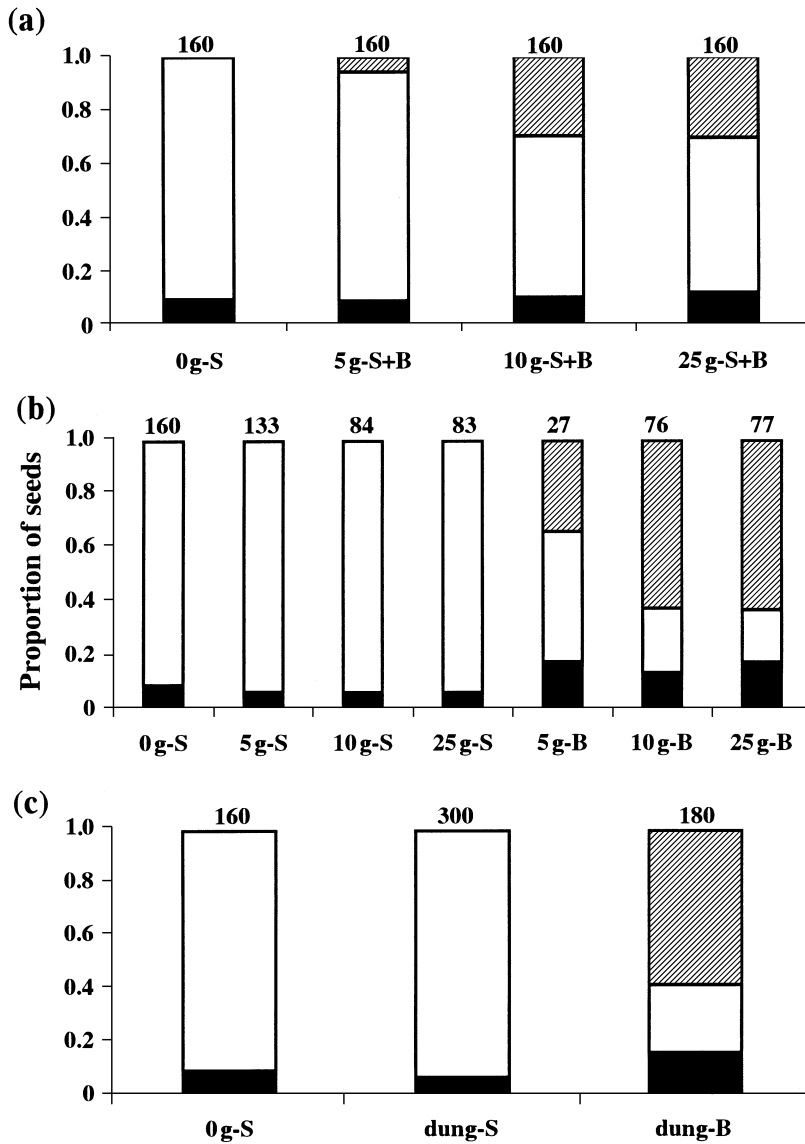


Figure 1. Proportion of seeds with the following fates: seedling establishment (filled), seed predation/removal (open), and seed emergence failure (hatched), (a) for the four initial dung treatments: 0, 5, 10 and 25 g of howler monkey dung (bars for 5, 10 and 25 g include both seeds remaining on the surface and seeds buried by beetles (S+B)); (b) for seeds buried by dung beetles (B) and seeds remaining on the surface (S), in each of the dung treatments; and (c) for the 5-, 10- and 25-g treatments pooled together (dung) for buried and surface seeds, compared to the 0-g treatment. Numbers above the bars indicate number of seeds.

repeated the analyses considering only seeds that had been buried by dung beetles. The results were the same. No statistically significant differences were found in the number of seedlings establishing from buried seeds with more dung, than with less dung ($\chi^2 = 0.46$, $df = 2$, $P > 0.5$). Seed predation/removal was lower in the 10- and 25-g treatments than in the 5-g treatment ($\chi^2 = 8.07$,

df = 2, $P < 0.05$), but the number of seeds that failed to emerge was higher for the 10- and 25-g treatments than for the 5 g treatment ($\chi^2 = 7.73$, df = 2, $P < 0.05$; Figure 1b, last three columns).

For seeds that remained on the surface, the proportion of seedlings establishing and the proportion of seeds predated/removed by rodents did not vary among dung treatments ($\chi^2 = 7.50$, df = 3, $P = 0.06$; Figure 1b, first four columns). However, the presence of dung did have an effect on seed predation/removal when measured 1 d after the seeds were placed on the forest floor, i.e. while some of the dung still remained on the soil surface. When comparing predation/removal of seeds placed with dung (pooling all dung treatments) but were not buried by dung beetles, with seeds placed without dung, predation after one day was significantly higher in the former group (23%) than in the latter (9%; $\chi^2 = 18.5$, df = 2, $P < 0.01$). Over time, however, as dung was quickly buried by dung beetles or washed away by rain, this effect disappeared. After 16 wk, the proportion of seeds remaining was the same in both groups of seeds ($\chi^2 = 0.32$, df = 2, $P = 0.57$; Figure 2). Although the number of remaining seeds also decreased over time for seeds buried by dung beetles, the proportion was always higher than for surface seeds, particularly when compared at the end of the study period ($\chi^2 = 289$, df = 1, $P < 0.01$; Figure 2).

As a consequence of the significantly lower predation/removal rate in seeds buried by dung beetles, and despite the high rate of emergence failure of such seeds, the proportion of seedlings establishing was significantly higher for

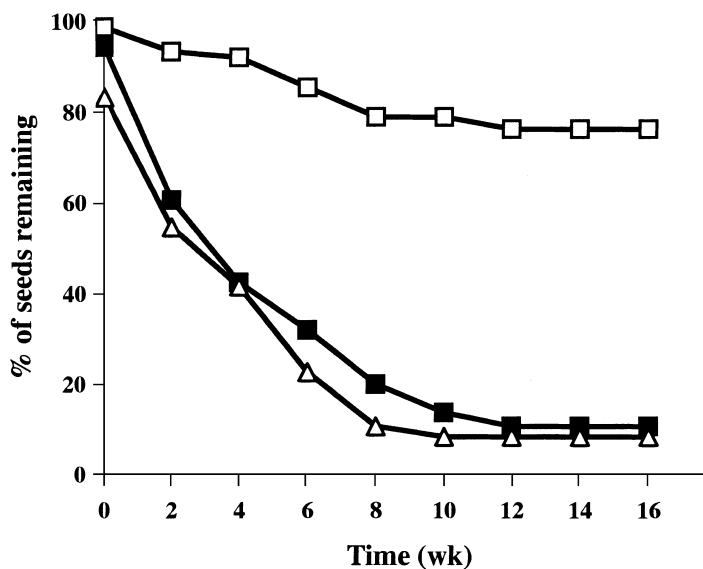


Figure 2. Percentage of seeds remaining over time, for seeds placed with howler monkey dung, and buried by dung beetles (open squares), seeds placed with dung and not buried by dung beetles (open triangles), and seeds placed without dung (filled squares). Time 0 represents 1 d after seeds were set out.

buried seeds than for surface seeds ($\chi^2 = 10.4$, $df = 2$, $P < 0.01$; after partitioning the contingency table, following Siegel & Castellan (1988): surface seeds with dung vs. surface seeds without dung, $\chi^2 = 0.78$, $df = 1$, $P > 0.05$; and, all surface seeds vs. buried seeds, $\chi^2 = 7.78$, $df = 2$, $P < 0.05$; Figure 1c).

Burial depth

The median burial depth for seeds surrounded by 10 g of dung was 5 cm (range: 0.5–14 cm, $n = 51$ seeds buried by beetles). Fifty-five per cent of seeds buried by dung beetles were at depths ≤ 5 cm, while 45% were buried at depths > 5 cm (Figure 3).

Twenty-two per cent of the seeds were moved horizontally by dung beetles. This movement could occur regardless of whether a seed was buried. Ninety-two per cent of seeds moved horizontally were found within 15 cm of the original location. The median horizontal distance was 7.5 cm and the maximum distance recorded was 80 cm.

Effect of burial depth on seedling establishment

Although all seeds placed at different depths in individual plastic cups with forest soil germinated, the percentage of seeds that established as seedlings decreased significantly with increasing burial depth (Cochran's test of linear trend: $\chi^2 = 7.58$, $df = 3$, $P < 0.01$; Pearson correlation coefficient = -0.971 ; Figure 4). At a depth of 5 cm only 49% of the germinating seeds were able to emerge, and at 10 cm only 11% emerged. Emergence failure of buried seeds was due to germinating seedlings not being able to break the soil column above them and hence dying.

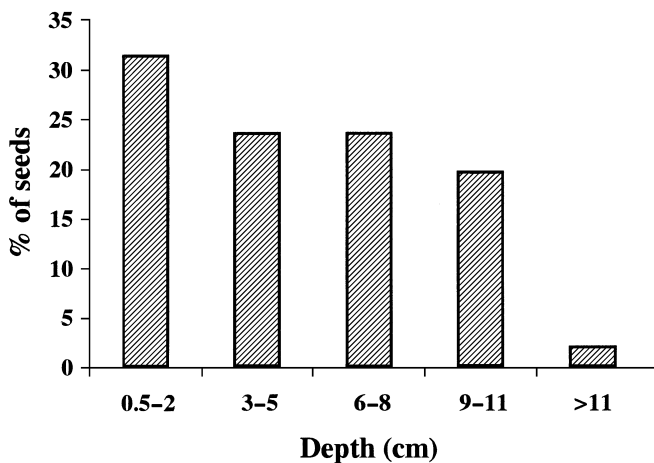


Figure 3. Percentage of *Micropholis* seeds surrounded by 10 g of howler monkey dung that were naturally buried in the field, by dung beetles, at different depths. A total of 51 seeds were buried by beetles out of 117 seeds used.

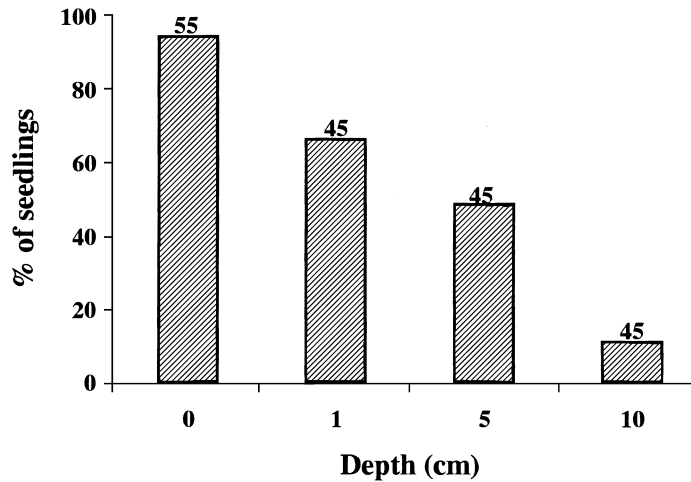


Figure 4. Percentage of seedlings establishing at different depths in controlled germination experiment (individual seeds in plastic cups). Numbers above bars indicate the number of seeds used for each depth treatment.

DISCUSSION

Presence of dung

The results of this study showed that the presence of dung around seeds dispersed through defecation by frugivorous mammals can be an important factor influencing the short-term fate of the seeds in the rainforest of Central Amazonia. However, although the initial fate of seeds was affected by the presence of dung, ultimately the same proportion of seedlings established from seeds surrounded by dung and seeds not surrounded by dung. This was because many of the seeds surrounded by dung did not get buried by beetles and suffered higher predation rates than surface seeds without dung and because many of the seeds buried by dung beetles were not able to emerge.

To what extent can this result be generalized? I repeated the experiment with seeds of 10 additional plant species, ranging from 11 to 26 mm in length. The lack of effect of the presence of dung on seedling establishment, when compared to seeds without dung, was found in nine of these species (Andresen 2000). Thus, for a plant that has its seeds dispersed by several means, producing a shadow of seeds in mammal dung (e.g. through defecation) and a shadow of dung-less seeds (e.g. through regurgitation by birds, spitting by mammals, scatterhoarding, gravity), both shadows will produce similar proportions of established seedlings, assuming that other aspects related to each dispersal mechanism are equal (e.g. treatment of seed, site of deposition, etc.).

However, when a plant species has its seeds mostly dispersed through mammal defecation, as is probably the case of *Micropholis* and other Sapotaceae species (Julliot 1996), it is advantageous for seeds to be buried by dung beetles, relative to remaining on the surface. Of the 10 additional seed species tested, six also showed a significant positive effect of seed burial, while the other four

did not show significant differences (Andresen 2000). Thus, it seems that in general, seeds buried by dung beetles have a higher probability of successfully establishing as seedlings than seeds on the surface.

Seed burial can be advantageous in several ways. One consequence of burial – and probably the most important for plants in tropical rainforests – is that buried seeds have a better chance of escaping detection by vertebrate and/or invertebrate seed predators (Andresen 1999, Crawley 1992, Estrada & Coates-Estrada 1991, Forget 1991, Hulme 1994, Shepherd & Chapman 1998, Vander Wall 1993a).

Another consequence of seed burial is that buried seeds encounter more moderate and less variable microclimatic conditions than seeds on the soil surface. Previous studies have shown that seed burial can prevent seed desiccation and promote germination (Forget 1991, Vander Wall 1993a, Wicklow *et al.* 1984) and may even help maintain viability of dormant seeds (Borchert *et al.* 1989). The seeds of some Sapotaceae species are prone to desiccation, which affects germination negatively (Benitez-Malvido 1995). However, the results of my germination experiment indicate that, at least for *Micropholis*, the moisture at soil-surface level is adequate for successful germination. The ‘microclimatic consequence’ of seed burial is very important in environments with extreme moisture and/or temperature conditions (Vander Wall 1993b, and references therein). Thus, in the context of tropical forests, this aspect of seed burial may be important in forests that show a stronger seasonality in precipitation and for plant species whose seeds have to survive and/or germinate during the dry season.

Amount of dung

The effect of amount of dung on the probability of seeds being buried by dung beetles has not previously been assessed, but it is likely to be of ecological importance. Almost 50% of *Micropholis* seeds surrounded by 10 or 25 g of dung were buried by dung beetles, while only 17% of seeds surrounded by 5 g of dung were buried by beetles.

The cause for this relationship is probably due, in part, to the fact that larger dung piles attract more dung beetles than do smaller dung piles (Andresen 2000, Peck & Howden 1984). Beetle size may also be of importance. Although I found no difference in the mean size of dung beetles captured with different-sized baits (Andresen 2000), other researchers have found that smaller baits attract beetles of mean smaller sizes (Peck & Howden 1984). Since a seed of a given size is proportionally a smaller contaminant for large beetles than for small beetles, the probability of the seed being buried will increase if handled by large beetles (Andresen 2000, Feer 1999).

The relationship between amount of dung and the probability of a seed being buried is ecologically important because it links secondary seed dispersal (movement of seeds by dung beetles) to primary seed dispersal (defecation of seeds by arboreal mammals). Different species of frugivorous mammals have

different patterns of defecation, characterized by the total amount of dung produced, the size distribution of faecal clumps and the spatial distribution of faecal clumps (Andresen 1999, Estrada *et al.* 1993, Zhang & Wang 1995). By knowing the type of defecation pattern produced by a frugivore, one will be able to assess the importance of secondary dispersal by dung beetles on the fate of the seeds dispersed by that frugivore. This may be a useful additional variable when comparing the quality of different primary dispersers, which traditionally have been compared only in terms of the number of seeds they disperse, the effect of passage through the animal gut, and the distance seeds are dispersed away from the parent plant (Schupp 1993, and references therein).

In the case of *Micropholis* seeds, although seeds were buried by dung beetles more often when surrounded by 10 and 25 g of dung, than when surrounded by 5 g of dung, this did not translate into an effect at the level of seedling establishment: the same proportion of seedlings established from all treatments. This was due to compensatory effects of seed predation and emergence failure. Buried seeds from the 10- and 25-g treatments suffered low predation rates but had high emergence failure; buried seeds from the 5-g treatments experienced the opposite. This was most likely due to seeds with more dung being buried more deeply than seeds with less dung. In an experiment in which I used plastic beads as seed mimics, placing them in 5-, 10-, and 25-g clumps, I found that beads surrounded by more dung were not only buried more often, but also more deeply (Andresen 2000). Such a compensatory effect may not exist for seeds or other plant species. For example, seeds that germinate rapidly may suffer lower predation pressures, and more seedlings may establish from seeds surrounded by less dung, and consequently buried less deeply, relative to seeds surrounded by more dung.

Burial depth and seedling emergence

Many studies have shown that the ability of seed predators to detect seeds diminishes with increasing burial depth (Andresen 1999, Estrada & Coates-Estrada 1991, Feer 1999, Shepherd & Chapman 1998, Vander Wall 1993b, and references therein). However, while deep burial has a positive effect on seed fate by decreasing probability of predation, it also has a negative effect by hindering the emergence of the germinating seeds. Studies on the germination and seedling establishment of temperate plant species have shown that an optimal range of depths exists for each species: while some seeds require very shallow burial depths, others do better at larger depths (see Vander Wall 1993a, and references therein). Very few studies, however, have addressed the effect of burial depth on germination and seedling establishment of rainforest tree seeds. Two studies have at least indirectly assessed this issue for tropical species with small seeds and both found that seedling establishment decreased with depth (Dalling *et al.* 1994, Fenner 1987). Two additional studies, one with four medium- to large-seeded tree species in Africa (Shepherd & Chapman

1998), and one with a large-seeded species in French Guiana (Feer 1999), yielded similar results.

In this study, *Micropholis* seedlings in the germination experiment established best when seeds were on the soil surface. Even seeds buried at 1 cm showed a considerable decrease in their ability to establish as seedlings. I performed this germination experiment with nine additional medium- to large-seeded rainforest tree species (9–25 mm seed length). Only two of these species showed a decrease in seedling establishment for surface seeds compared to seeds buried at 1 cm. In the case of buried seeds, all species showed a negative effect of increasing depth on seedling emergence (Andresen 2000).

Comparison with other studies

Although in general the results of this study agree with those of the previous studies on seed dispersal by dung beetles (Andresen 1999, Estrada & Coates-Estrada 1991, Feer 1999, Shepherd & Chapman 1998), it is interesting to note some discrepancies and to try to identify some of their causes. Most of the differences in these studies occur in terms of percentage of seeds buried by dung beetles, and in terms of burial depths (for seeds of similar dimensions). Surely the most important factor in determining whether secondary dispersal by beetles occurs for comparable seeds in different sites, is the dung beetle community present in a site. Dung beetle communities vary from one rain forest area to another, even within relatively similar regions (e.g. in different regions of the Amazon Basin; Vulinec 1999; E. Andresen, *pers. obs.*). But also within a given forest site several variables can affect the species and number of beetles that are attracted to dung in a given moment, and consequently affect secondary dispersal of seeds in dung. Such variables include season of the year (wet vs. dry), time of day (day vs. night), and amount of dung (Andresen 2000, Hanski & Cambefort 1991a). For example, in tropical forests with a pronounced seasonality in rainfall, adult beetles show lower abundance or may even disappear during the dry season (Janzen 1983): secondary dispersal by dung beetles is likely non-existent during the dry season in such forests.

Other causes for differences among the results of studies on seed dispersal by dung beetles include methodological aspects. For example, I think it is highly important to use fresh dung in experiments and beetle captures. Dung that is a few days old, even if kept in plastic bags, may lose its attractiveness to dung beetles and consequently affect secondary seed dispersal. Also, percentage of seed burial and burial depth have often been measured in experiments in which dung beetles buried the seed-containing dung presented on top of plastic cylinders filled with soil (Andresen 1999, Estrada & Coates-Estrada 1991, Feer 1999, Vulinec 1999). The horizontal space around the dung source is limited in such cylinders. Since competition for dung is severe among beetles (Hanski & Cambefort 1991d), a restricted space availability may affect their dung-burying behaviour. Also, the soil placed in the cylinder is likely to be less compacted than soil on the forest floor, and it is known that soil characteristics

can significantly affect the dung-burying behaviour of some dung beetles (Fincher 1973).

Concluding remarks

Secondary dispersal of seeds occurs in many dispersal systems, and although less studied than primary dispersal, it is becoming evident that this phase can add great complexity to the seed dispersal ecology of plants (Andresen 1999, Böhning-Gaese *et al.* 1999, Chambers & MacMahon 1994, Estrada & Coates-Estrada 1991, Forget *et al.* 1998, Levey & Byrne 1993, Wenny 1999).

How common is secondary dispersal by dung beetles? Dung beetles are often very abundant in tropical forests (Hanski & Cambefort 1991a), and the dung they preferentially use in most regions of the world is that of large herbivorous vertebrates (Hanski 1991). Mammals, constituting the largest proportion in the biomass of herbivorous vertebrates in tropical forests, probably produce most of the dung available to dung beetles in these areas. And, since in many tropical rain forests most mammal species have at least partially frugivorous diets (Terborgh 1986), it is likely that much of the dung that dung beetles are attracted to, in these ecosystems, will contain seeds in it. Thus, the potential for secondary dispersal by dung beetles is large, and their role should be taken into consideration when assessing the effect that a mammalian primary disperser has on the regeneration of a plant species whose seeds it defecates.

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