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The Effects of Large Terrestrial Mammals on Seed Fates, Hoarding, and Seedling Survival in a Costa Rican Rain Forest

Erin K. Kuprewicz

University of Miami, erin@bio.miami.edu

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UNIVERSITY OF MIAMI

THE EFFECTS OF LARGE TERRESTRIAL MAMMALS ON SEED FATES,
HOARDING, AND SEEDLING SURVIVAL IN A COSTA RICAN RAIN FOREST

By

Erin K. Kuprewicz

A DISSERTATION

Submitted to the Faculty
of the University of Miami
in partial fulfillment of the requirements for
the degree of Doctor of Philosophy

Coral Gables, Florida

May 2010

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Erin K. Kuprewicz

Approved:

Michael S. Gaines, Ph.D.
Professor of Biology

Terri A. Scandura, Ph.D.
Dean of the Graduate School

David Janos, Ph.D.
Professor of Biology

Theodore Fleming, Ph.D.
Professor Emeritus of Biology

Patrick Jansen, Ph.D.
CTFS-SIGEO Vertebrate Program Coordinator
Smithsonian Tropical Research Institute
Panama

KUPREWICZ, ERIN

(Ph.D., Biology)

The Effects of Large Terrestrial Mammals on Seed
Fates, Hoarding, and Seedling Survival in a Costa
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Terrestrial mammals affect numerous aspects of plant demography, colonization, and community structure in Neotropical forests. Granivorous mammals destroy seeds via seed predation and seedlings through herbivory, negatively affecting plant fitness.

Mammals can also positively affect plants by dispersing or hoarding seeds. Seed fate outcomes are contingent on the interaction between mammal seed handling strategies and the intrinsic anti-predation defenses possessed by seeds. In field experiments at La Selva Biological Station, I investigated how collared peccaries (*Pecari tajacu*) and Central American agoutis (*Dasyprocta punctata*) affect five species of large seeds that have various defenses against predation. Overall, peccaries consumed and killed most non-defended and chemically-defended seeds but they could not destroy seeds with physical defenses. Agoutis killed non-defended and physically-defended seeds, but not seeds with chemical defenses.

Using seeds of *Mucuna holtonii*, I investigated how chemical and structural defenses deter mammal and insect seed predation respectively. I also determined how endosperm removal by invertebrates affects seed germination and seedling biomass. Chemical defenses protected seeds from rodents, but not ungulates that digest seeds via pregastric fermentation. Physical defenses protected seeds from invertebrate seed

predators, and removal of endosperm negatively affected both seed germination and seedling growth.

To determine how scatter-hoarding by agoutis affects seed escape from seed predators, germination, and seedling growth, I created simulated agouti hoards. I also investigated how mammals affect young seedling survival. Hoarding enhanced seed survival, germination, and seedling growth for most species of seeds. Terrestrial mammals killed some seedlings via seed predation rather than by herbivory. Overall, large mammal activity in La Selva negatively affected seed and seedling survival and this likely influences many aspects of forest dynamics.

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CHAPTER 1

Seed predation, dispersal, and hoarding by large neotropical mammals: impacts on seed and seedling survival

BACKGROUND

Terrestrial mammals play a major role in the predation of seeds within Neotropical forests (*e.g.* Brewer and Rejmánek 1999, DeMattia 2004, Forget 1996, Janzen 1971c, Silman *et al.* 2003). Mammal-mediated seed predation has profound effects upon plant recruitment and forest community structure. Mammals either completely or partially consume large seeds and this predation usually leads to seed death. Seed predation positively affects seed predators but negatively affects plants. However, the interactions between mammal seed predators and the large seeds they consume become more complex as extrinsic factors and intrinsic traits of seed-eating mammals and seed species are considered.

To prevent seed losses and consequent reductions in fitness, some plants have evolved protective strategies against seed predators in the form of physical defenses (*e.g.* spines, thick fruit endocarps) or chemical defenses (*e.g.* secondary compounds that render seeds toxic to many seed predators) (Bell 1984, Bodmer 1991, Dirzo and Dominguez 1986, Janzen 1971c, Kiltie 1982). The presence and intensity of seed defenses varies among plant species and may not completely protect seeds from destruction by all seed-eating animals within a forest (Janzen 1971c, Kuprewicz and García-Robledo 2010). Intrinsic anti-predation seed defenses interact with mammal seed-handing strategies to produce variable outcomes in seed fate and survival.

Although granivorous mammals may consume and destroy a high proportion of a plant's seed crop, seed predators may also disperse some seeds (*e.g.* Bodmer 1991, Howe and Smallwood 1982). Mammals may possibly provide high quality, reliable dispersal to some plant species thereby enhancing seed survival and seedling recruitment (*e.g.* Hallwachs 1986, Janzen 1971c). Seed dispersal is considered a major ecological force in plant recruitment and in the structuring of forest communities (Fenner and Thompson 2005, Howe and Smallwood 1982). Vertebrates disperse approximately 70% - 90% of woody plant species in tropical forests and of these animals, mammals act as major seed dispersal agents (Fleming *et al.* 1987). Terrestrial mammals can disperse large seeds via endozoochory, ectozoochory, or hoarding. Endozoochorous dispersal of large seeds involves a seed being swallowed, passed intact through an animal's gut, and ultimately deposited in feces some distance from the parent plant (Beck 2005, Travaset *et al.* 2007). Mammals can also disperse seeds by carrying them in their mouths and spitting unpalatable, too large, or unbreakable seeds onto the forest floor as one form of ectozoochory. Some rodents also disperse seeds by burying them below the soil in subsurface caches in a process called hoarding (*e.g.* Jansen *et al.* 2004, Smythe 1978).

Granivores hoard seeds in order to conserve them for future use. Consumption of a hoarded seed is temporally deferred and it is transported, deposited, and concealed by the hoarder to prevent detection by other seed-eating animals (Vander Wall 1990). Animals that hoard plant propagules may serve as incidental dispersers of their intended food items if seed-hoarding animals fail to relocate cached seeds and seeds germinate and become seedlings. Scatter-hoarding mammals bury single seeds in shallow caches spaced throughout their home ranges (Gálvez *et al.* 2009, Vander Wall 1990)

These caches not only hide plant propagules from predators, but also provide potentially favorable microsites for plant establishment (Forget 1990, Jansen *et al.* 2004, Vander Wall 1990).

Seeds hoarded by mammals benefit from this form of seed dispersal in numerous ways. One benefit conferred to hoarded seeds is that they are transported from beneath the parent plant where seed predation pressure and competition are high (*e.g.* Howe and Smallwood 1982, Janzen 1970). Seed handling by mammals prior to hoarding may increase the germination success of cached seeds (Vander Wall 1990). Scatter-hoarding by mammals also effectively hides buried seeds from invertebrate and vertebrate seed predators that would otherwise destroy seeds on the soil surface (Borchert *et al.* 1989, Vander Wall 1990). Finally, hoarded seeds have a higher likelihood of germinating than seeds located aboveground if they are located in a microhabitat favorable to germination and seedling growth (*e.g.* Vander Wall 1990 and references therein).

Seeds and seedlings are particularly susceptible to destruction by generalist frugivore-granivores and this has profound effects on adult tree distributions and forest dynamics (*e.g.* Connell 1971, Janzen 1970, Silman *et al.* 2003). Large terrestrial mammals can destroy young seedlings in the understory through herbivory (Asquith *et al.* 1997, Clark and Clark 1989, DeMattia *et al.* 2006), trampling while foraging (*e.g.* Clark and Clark 1989), or predation of the seed still attached to the growing seedling (Brewer and Webb 2001, Pyare and Longland 2000, Smythe 1989).

Interactions of peccaries and agoutis with large seeds and seedlings in La Selva follow a general pattern with variable potential outcomes as illustrated in the seed fate diagram in Figure 1.1.

STUDY SITE

I conducted this research at the La Selva Biological Station (hereafter La Selva), Puerto Viejo Sarapiquí, Heredia, Costa Rica (10° 26' N, 83° 59' W) (Figure 1.2). La Selva contains approximately 1,600 ha of tropical wet forest and disturbed land with 55% of the property classified as old-growth (primary) forest, the forest type in which I conducted most of the following experiments. Rainfall at this site averages approximately 4,000 mm per year with no dry season (each month receives on average > 100 mm of rainfall). The forest within La Selva is characterized as lowland tropical wet forest (Holdridge *et al.* 1971). See McDade *et al.* (1994) for a detailed description of this site. La Selva contains a unique mammal community making it an especially amenable site for my research. Although the reserve is connected via a corridor to Braulio-Carrillo National Park (47,753 ha), hunting and fragmentation in the areas surrounding La Selva have resulted in the local extinction of some large mammal species (*e.g.* white-lipped peccaries, *Tayassu pecari*) within La Selva. La Selva houses large local populations of collared peccaries (*Pecari tajacu*) and Central American agoutis (*Dasyprocta punctata*), probably because of the local rarity of predators (*e.g.* large felids) and high year-round food availability from fruiting trees (especially palms).

STUDY SPECIES, MAMMALS

Central American agouti (*Dasyprocta punctata* Gray, 1842)

The Central American agouti (*D. punctata*, Rodentia: Dasyproctidae) is a large (Head and body length = HB: 45 - 57 cm; Weight = Wt: 3 - 4 kg) terrestrial forest-dwelling rodent (Reid 1997) that ranges from Chiapas and Campeche, Mexico, through

Central America to northwest Colombia and Ecuador, west of the Andes (Emmons 1997). Agoutis are diurnal and occupy territories in monogamous pairs, but each adult usually forages alone throughout the day. *Dasyprocta punctata* are generalist frugivore-granivores that consume a wide variety of fruits and, more rarely, fungi, leaves, flowers, and insects (Smythe 1978). Agoutis are habitat generalists that inhabit a variety of forest types from mature and secondary lowland and montane forest to tropical dry forest and plantations.

The Central American agouti is the largest extant scatter-hoarder. Agoutis bury large seeds singly in shallow caches under the soil (2 - 8 cm deep) for retrieval when fruit is scarce. These mammals may serve as the primary (or only) seed dispersers for some large-seeded tropical trees (*e.g. Hymenaea courbaril* and *Astrocaryum* spp.) (Hallwachs 1986, Smythe 1989). This species is of potential conservation concern because some populations are threatened and others have gone locally extinct due to over-hunting and habitat loss through deforestation (Forget and Jansen 2007). Also, due to their tolerance of disturbed habitats, *D. punctata* may serve as potential natural reforestation agents by dispersing large seeds from intact habitat to disturbed habitat.

Collared peccary (Pecari tajacu Linnaeus, 1758)

The collared peccary (*P. tajacu*, Artiodactyla: Tayassuidae) is a large (HB: 80 - 100 cm; Height at shoulder = SH: 40 - 50 cm; Wt: 12 - 30 kg) terrestrial mammal that ranges throughout Middle and Central America to northern South America (Reid 1997). Collared peccaries consume fruits and seeds as the bulk of their diet and they also eat other plant and animal materials (Beck 2005). *Pecari tajacu* forage in herds ranging

from 2-50 individuals that travel over large home ranges (*e.g.* 118 ha in a Costa Rican dry forest, McCoy et al. 1990). These large frugivores comprise the majority of mammal biomass in many neotropical forests. Due to their large body masses and herd sizes, these animals act as major ecosystem engineers since they perturb soil, destroy seedlings (via trampling and herbivory), and they consume and kill seeds during their intense daily foraging activities. As seed predators, peccaries play a significant role in seed survival to germination and subsequent seedling recruitment (*e.g.* Asquith et al. 1997).

Pecari tajacu not only consume seeds that they encounter on the forest floor during routine foraging bouts, but these animals also act as kleptoparasites, seeking out, unburying, and stealing food stored in shallow underground caches by scatter-hoarding agoutis (*pers. obs.*). While scatter-hoarding effectively aids in seed dispersal, the kleptoparasitic behavior of peccaries negates any positive effects a hoarded seed gains by agouti-mediated dispersal.

STUDY SPECIES, PLANTS

I used fresh, ripe seeds and young seedlings of *Astrocaryum alatum*, *Iriartea deltoidea*, *Socratea exorrhiza* (all Arecaceae), and entire fruits of *Dipteryx panamensis* (Fabaceae: Papilionoideae) for seed fate, hoarding, and seedling survival experiments. I also included *Mucuna holtonii* (Fabaceae: Papilionoideae) seeds in seed fate experiments (these seeds are not hoarded by agoutis, therefore I did not use them in hoarding or seedling experiments). *A. alatum*, *I. deltoidea*, *S. exorrhiza*, and *D. panamensis* trees are locally abundant throughout the primary forest of La Selva. These fruits and seeds are readily hoarded by agoutis and eaten by agoutis, peccaries, squirrels, and small rodents.

Mucuna holtonii lianas are found throughout the secondary forest habitat of La Selva (and in some parts of primary forest) but these seeds are not hoarded by agoutis and are only eaten by mammals that can contend with *M. holtonii* seeds' secondary compounds. Due to large seed sizes, agoutis and peccaries do not pass these five diaspores through their guts intact (endozoochory), rather they grind and crush seeds prior to ingesting them resulting in complete seed destruction and death.

Astrocaryum alatum

Astrocaryum alatum H. F. Loomis is a 1.5 - 7 m tall 10 - 17 cm diameter palm covered with large flattened black spines covering the stem and leaves (Henderson *et al.* 1995). *Astrocaryum alatum* are found in lowland rain forests on the Atlantic slopes in Nicaragua, Costa Rica, and Panama to the Panama Canal, and the Pacific slope in Costa Rica. This species produces large obovoid one-seeded fruits on a pendulous infructescence (25-50 fruits); individual fruits are covered with a spiny, firm yellow-brown exocarp (0.32 cm thick) that splits when ripe to reveal a thin white mesocarp; the enclosed seed (length = 6 cm, width = 4 cm) is covered with a thick (5 mm) stony brown endocarp and has homogenous endosperm with a hollow center.

Iriartea deltoidea

Iriartea deltoidea Ruiz & Pav. is a 25 m tall 30 cm diameter palm supported by a dense mass of spiny black stilt roots; this species ranges from Nicaragua south to Bolivia, extending into the western Amazon (Henderson *et al.* 1995). Adult *I. deltoidea* trees produce dark green to black single-seeded globoid fruits comprised of a thin exocarp

covering a spongy white mesocarp that surrounds the seed. The spherical seeds (2 - 2.8 cm diameter, Henderson *et al.* 1995) have homogenous endosperm and are readily eaten by toucans and monkeys in the canopy and by peccaries and terrestrial rodents on the forest floor (pers. obs.).

Socratea exorrhiza

Socratea exorrhiza (Mart.) H. Wendl. is an 8 - 20 m tall 12 - 18 cm diameter palm supported by spiny brown stilt roots arranged diffusely around the tree's base. *Socratea exorrhiza* is commonly found in lowland and premontane rain forests from Nicaragua to Bolivia and northeastern South America (Henderson *et al.* 1995). The fruits (2.5 - 3.5 cm long, 1.5 - 2 cm diameter) of *S. exorrhiza* comprise dark brown exocarp that splits open at maturity to reveal a spongy white mesocarp surrounding a hard ovoid seed with homogenous endosperm. Fruits in the canopy are removed and eaten by bats, birds, and arboreal mammals; peccaries, agoutis, and small rodents consume *S. exorrhiza* seeds that fall to the ground (pers. obs.).

Dipteryx panamensis

Dipteryx panamensis (Pittier) Record & Mell is a large up to 40 m tall emergent canopy tree found in lowland wet tropical forests ranging from Nicaragua to Colombia (Flores 1992). *Dipteryx panamensis* trees produce single-seeded drupaceous, oleaginous fruits (length = 6 cm, width = 3 cm) comprised of a spongy exocarp surrounding a thick, stony endocarp that encases the seed (length = 5 cm, width = 1.5 cm). Upon germination, the suture encircling the exocarp dehisces to allow for radicle and plumule emergence.

Mucuna holtonii

Mucuna holtonii (Kuntze) Moldenke is a liana found throughout tropical rain forests from Chiapas, Mexico to Colombia. *Mucuna holtonii* lianas are most common in secondary forest, but can also be found within primary forest habitat (Woodson Jr. and Scherry 1980). The pendulous infructescences of *M. holtonii* comprise 1-9 flat, oblong legumes (length = 14-25 cm, width = 5 cm). Each *M. holtonii* fruit pod contains 1-6 black discoid seeds (diameter = 2-3 cm, thickness = 0.8 cm) (Woodson Jr. and Scherry 1980). Upon maturation, *M. holtonii* legumes dehisce and the mature seeds fall to the forest floor where they may be encountered by terrestrial vertebrates and invertebrates. Seeds of this legume contain high concentrations of the toxic amino acid L-dopa, which deters predation by some seed-eating animals (Daxenbichler *et al.* 2000, Rehr *et al.* 1973, Janzen *et al.* 1973, VanEtten *et al.* 1972).

OBJECTIVES

The main objectives of my dissertation were to elucidate patterns and processes involving abundant mammal seed predators, large seeds, and seedlings within a tropical rain forest in Costa Rica. More specifically, I determined how terrestrial mammal abundances, seed handling strategies, and seed defenses interact to affect seed fates and seed survival outcomes (Chapter 2). I also assessed if chemical and structural defenses in *M. holtonii* seeds prevent predation by mammal and insect seed predators and how insect attack impacts seed germination and subsequent biomass production (Chapter 3). Finally I quantified the positive and negative impacts of seed scatter-hoarding by agoutis on seed

escape from predators (vertebrates and invertebrates), seed germination, seedling growth, and the subsequent effects of mammals on young seedling survival (Chapter 4).

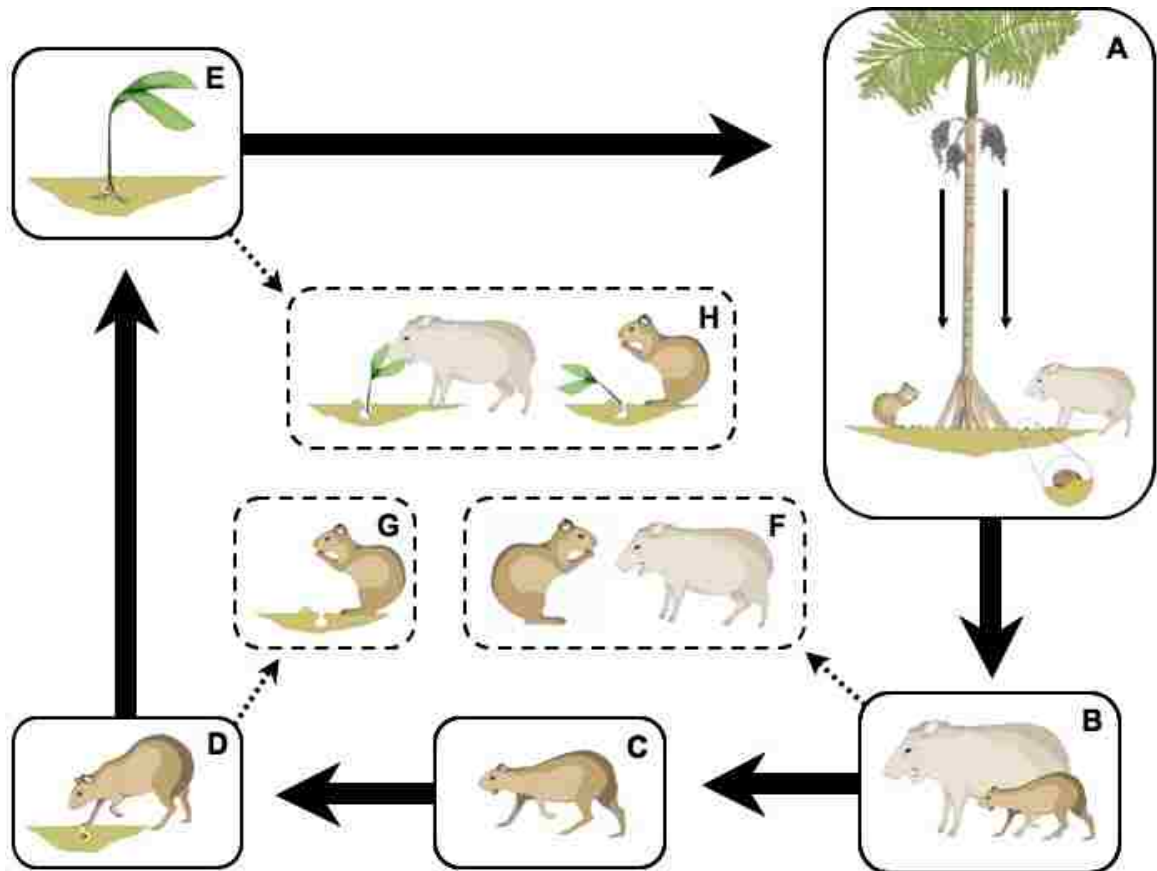


Figure 1.1. Generalized seed fate diagram involving seeds, seedlings, agoutis, and peccaries in La Selva. Solid arrows indicate non-predation events and seed/seedling survival; dashed arrows indicate predation events and seed/seedling death. Seeds fall from a parent tree and are encountered by agoutis and peccaries on the ground (A). Seeds are removed from beneath the parent tree by agoutis and peccaries (B). Agoutis remove seeds long distances (C) before burying a seed in a subsurface cache (D, hoarding). If the seed remains intact in an agouti cache, it may germinate and become a seedling (E) whereupon it may grow into an adult fruit-bearing plant and the cycle begins anew. Seeds removed from the source tree can be dispersed but eventually eaten by agoutis and peccaries (F). Hoards can be excavated and the seed eaten (G). Seedlings can be dug up and killed by peccaries and agoutis (H).

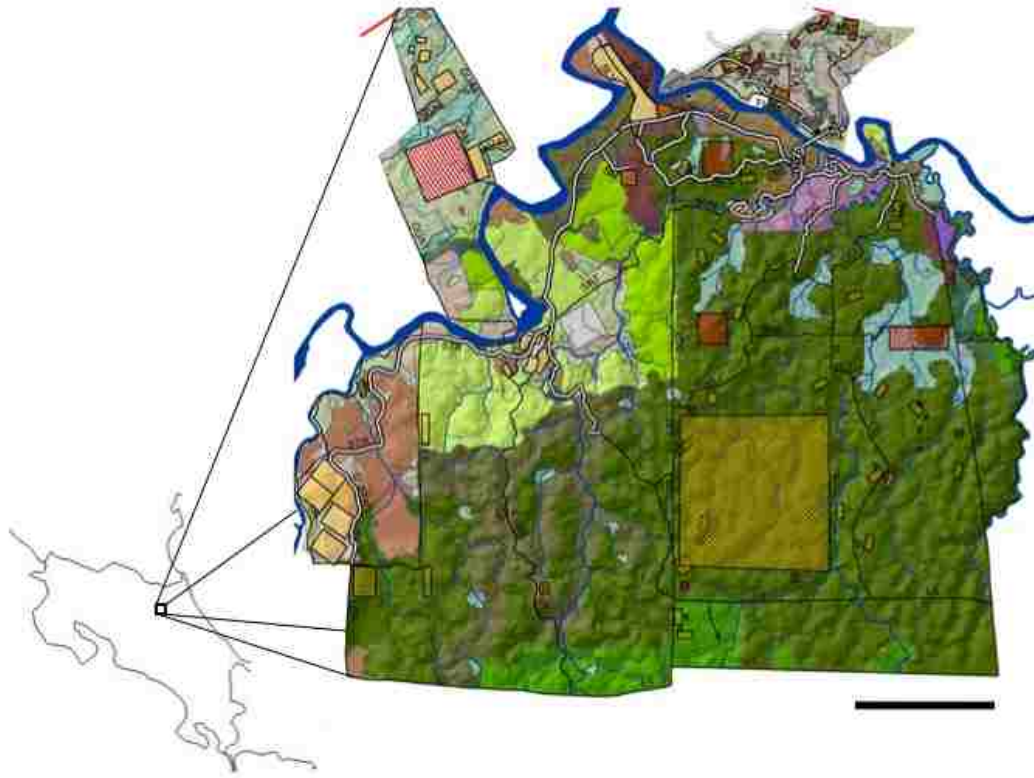


Figure 1.2. Map of my study area: La Selva Biological Station, Costa Rica, Central America. Scale bar = 1000 m.

CHAPTER 2

Relative terrestrial mammal abundances and contrasting seed defense strategies affect neotropical seed fates

SUMMARY

In Neotropical forests, mammals are major seed dispersers and predators. To prevent seed predation and promote dispersal, seeds may exhibit physical or chemical defenses. Collared peccaries (*Pecari tajacu*, Artiodactyla) cannot eat some hard seeds, but can digest many chemically-defended seeds. Central American agoutis (*Dasyprocta punctata*, Rodentia) gnaw through hard-walled seeds, but cannot consume some chemically-defended seeds. The goals of this study were to determine relative peccary and agouti abundances within a lowland wet forest in Costa Rica and to assess how these mammals affect the survival of seeds that have no defenses (*Iriartea deltoidea*, *Socratea exorrhiza*), physical defenses (*Astrocaryum alatum*, *Dipteryx panamensis*), or chemical defenses (*Mucuna holtonii*) against seed predators. I determined relative mammal abundances over three years using motion-detecting camera trap data. Using semi-permeable mammal exclosures, camera traps, and thread-marked seeds, I recorded predation and dispersal for each seed species. Relative abundances of peccaries were up to 6x higher than those of agoutis from 2006 – 2008. Non-defended seeds and chemically-defended seeds suffered high levels of predation, mostly by peccaries. Only 23% of physically-defended seeds were eaten by agoutis; seeds of *A. alatum* benefited from dispersal by peccaries. Peccaries did not eat seeds of *A. alatum*. Peccaries and agoutis did not differ in the distances they dispersed seeds. This study shows that the

effectiveness of physical and chemical seed defenses against seed predation depends on the relative abundances and seed handling capabilities of mammalian frugivore-granivores.

BACKGROUND

Animal-mediated seed dispersal is a fundamental process that can potentially affect seedling establishment, tree distributions, and the maintenance and regeneration of forest ecosystems (Curran *et al.* 1999, DeMattia *et al.* 2004, Forget 1996, Fragoso 1997, Howe and Brown 2001, Peres and Baider 1997). In tropical forests, mammals play a major role in seed dispersal and predation. Large-bodied terrestrial mammals may disperse seeds by transporting and depositing them either on the soil surface or by burying them in subsurface caches (scatter-hoarding) (Vander Wall 1990). Dispersed seeds may germinate if they are incidentally or intentionally deposited in suitable microhabitats by mammalian frugivores. However, granivorous mammals also consume and destroy many of the seeds they encounter while foraging, acting as seed predators (Asquith *et al.* 1997, DeMattia *et al.* 2004, Jansen *et al.* 2004, Kuprewicz Chapter 4 in this dissertation, Kuprewicz and García-Robledo 2010).

Although terrestrial mammals affect neotropical trees positively, via dispersal and hoarding, and negatively, through predation, herbivory, and seedling trampling, little is known about the actual net effects that mammals have on seed survival. This scenario can be even more complex because the effect of terrestrial mammals on different plant life stages may vary among mammal and plant species. Neotropical mammals vary in their abilities to consume and process fruits and seeds due to differences in physiological

characteristics such as jaw strength (Kiltie 1982), eating stance, or digestive physiology (Desbiez *et al.* 2009). For example, collared peccaries (*Pecari tajacu* Linnaeus, 1758) cannot masticate and consume some seeds with hard, thick endocarps, whereas Central American agoutis (*Dasyprocta punctata* Gray, 1842) hold seeds in their forepaws and can gnaw through thick-walled fruits to extract endocarp (Smythe 1978, Beck 2005, pers. obs.). Alternately, peccaries possess pregastric fermentation, which allows them to digest toxic seeds that other mammals cannot (Beck 2005, Kuprewicz and García-Robledo 2010).

Reciprocal selection pressures exist between seed-bearing plants and granivores (Janzen 1980). Plants have evolved seed defenses to deter predation and to enhance seed dispersal. These protective strategies include physical defenses such as spines or thick fruit endocarps that serve as deterrents or barriers to seed consumption, and chemical defenses that render seeds toxic or unpalatable to seed predators (Janzen 1971b, Kiltie 1982, Bell 1984, Dirzo & Dominguez 1986, Bodmer 1991, Kuprewicz and García-Robledo 2010). Some mammals can circumvent seed defenses and consume seeds despite physical or chemical protection, however granivore species vary in their intrinsic abilities to cope with seed defenses (Janzen 1971c).

Disparity in relative local frugivore abundances is an extrinsic factor that affects seed encounter rates and seed fate outcomes that are dependent on intrinsic frugivore seed handling strategies. Abundances and distributions of terrestrial mammal species vary throughout neotropical forests and this may affect seed fates within these habitats. Habitat variation, fruiting tree phenologies, food resource patchiness, predator abundances, home range requirements, and anthropogenic effects interact to produce

differences in terrestrial frugivorous mammal abundances within forests (Emmons 1984, Robinson and Redford 1986, Voss and Emmons 1996). Hunting and habitat fragmentation particularly affect distributions and abundances of large-bodied terrestrial frugivores (Wright *et al.* 2000). Understanding the roles large mammals play in plant reproductive processes is essential since these animals are detrimentally affected by deforestation and hunting by humans (Stoner *et al.* 2007). Large mammal declines can affect both intact and degraded forests that would potentially benefit from natural reforestation by mammal seed dispersers (Corlett and Hau 2000). Abundant mammal species that forage in large groups are most likely to encounter seeds and, depending on intrinsic seed handling strategies, may positively or negatively affect seed survival. Dependent on a mammal's foraging and seed handling strategy and combined with seed anti-predation defenses, an encountered seed may be eaten, dispersed, or hoarded; this outcome is determined by the interacting mammal and seed species.

If a seed is removed from its initial location, it may be dispersed. However, seed dispersers vary in the distances they tend to move seeds (Willson and Traveset 1992). One qualitative measure of seed dispersal effectiveness (*sensu* Schupp 1993) is seed dispersal distance. Seeds benefit from long-distance dispersal by avoiding intense predation pressure near parent plants, decreasing potential competition with conspecifics, and possibly locating microhabitats favorable to seed germination and seedling growth (Howe and Smallwood 1982 and references therein). Terrestrial mammal species vary in their likelihoods of dispersing seeds and also in the distances they transport intact seeds. Mammals that have large home ranges or large foraging areas will tend to disperse seeds farther than mammals that have small home ranges (Fragoso *et al.* 2003). Distances

seeds are dispersed by mammals also depend on interactions between intrinsic seed consumption behaviors by animal dispersal agents and seed defense strategies employed by plants.

In this seed fate study, I tested the following hypotheses and predictions: (1) Seeds on the forest floor are encountered first by peccaries rather than agoutis due to peccaries' high levels of local abundance and group foraging behavior. (2) Seed survival depends on the interaction of mammal seed-handling characteristics and seed defenses; more non-defended and chemically-defended seeds exposed to peccaries will be destroyed when compared to seeds exposed only to agoutis because peccaries are abundant ungulates that can process toxic seeds via foregut fermentation. More physically-defended seeds will be destroyed by agoutis than by peccaries because agoutis can gnaw through thick endocarps that collared peccaries cannot penetrate. I will also test two alternate hypotheses concerning mammal seed removal and dispersal kernels: (1) peccaries incidentally move seeds they cannot consume by expectoration farther than agoutis because peccaries cover larger daily foraging ranges, or (2) agoutis move seeds they plan to hoard (directed dispersal) farther from sources than peccaries that incidentally disperse seeds via expectoration; agoutis move hoarded seeds far from sources to escape intense seed predation pressure near the source and to prevent kleptoparasitism of seeds by other granivores (*e.g.* peccaries).

METHODS

Study site

I conducted this study from September 2006 to December 2008 at La Selva Biological Station (henceforth La Selva) located in Puerto Viejo Sarapiquí, Heredia, Costa Rica (10°26' N, 83°59' W). La Selva is a tropical lowland wet forest that contains approximately 1600 ha of primary forest, secondary forest, swamps, and tree plantations. La Selva receives approximately 4000 mm of rainfall *per annum* and has no distinct dry season (McDade *et al.* 1994). Hunting in and habitat fragmentation of the land surrounding La Selva have resulted in the local extinction of some large terrestrial mammals (e.g. white-lipped peccaries, *Tayassu pecari* Link, 1795) in the region. For a detailed description of La Selva, see chapter 1 of this dissertation or McDade *et al.* 1994. I conducted the following study throughout La Selva's primary forest.

Study species -- mammals

Collared peccaries (*Pecari tajacu*, Artiodactyla) and Central American agoutis (*Dasyprocta punctata*, Rodentia) are the most abundant terrestrial mammal frugivores within La Selva (Kuprewicz and García-Robledo 2010, TEAM Network <http://www.teamnetwork.org/en/>). Collared peccaries are large (up to 30kg), terrestrial mammals that consume fruits and seeds as the bulk of their diets (Beck 2005). *Pecari tajacu* range throughout Central American and forage in herds ranging from 2-50 individuals that travel over large home ranges (e.g. 118 ha in a Costa Rican dry forest, McCoy *et al.* 1990). Due to their large body masses and herd sizes, peccaries act as major ecosystem engineers since they perturb soil, destroy seedlings (via trampling and

herbivory), and consume and kill seeds during intense daily foraging activities (Kiltie 1982, Beck 2005). As seed predators, peccaries play a significant role in seed survival to germination and subsequent seedling recruitment (Asquith *et al.* 1997). Peccaries cannot eat seeds protected by thick-walled endocarps, but due to foregut fermentation, they can digest many chemically-defended seeds (Kuprewicz and García-Robledo 2010).

Central American agoutis are large (3 - 4 kg) terrestrial frugivores ranging from southern Mexico through Central America south to northern Argentina (Reid 1997). *Dasyprocta punctata* are frugivore-granivores that consume, and potentially disperse, a wide variety of fruits and seeds (Smythe 1978). Agoutis bury large seeds singly in shallow caches under the soil for retrieval when fruit is scarce and they may serve as the primary seed dispersers for some large-seeded tropical trees (*e.g.* *Hymenaea courbaril* and *Astrocaryum* spp.) (Hallwachs 1986, Smythe *et al.* 1986). Agoutis can gnaw through hard-walled seeds, but may not be able to consume chemically-defended seeds (Kuprewicz and García-Robledo 2010).

Study species -- plants

For all seed fate experiments, I used fresh, ripe seeds of *Iriartea deltoidea* (Arecaceae), *Socratea exorrhiza* (Arecaceae), *Astrocaryum alatum* (Arecaceae), *Mucuna holtonii* (Fabaceae), and entire fruits of *Dipteryx panamensis* (Fabaceae) (Table 2.1). Hereafter I refer to seed fates and seed dispersal for all plant species, however the unit of dispersal for *D. panamensis* is the entire fruit (exocarp, endocarp, and seed). Due to the large sizes of these five seed species, peccaries and agoutis do not pass these diaspores through their guts intact (endozoochory), rather they grind up seeds prior to ingesting

them; predation of these seed species by peccaries and agoutis results in complete seed destruction. *Iriartea deltoidea* and *S. exorrhiza* seeds do not possess apparent protection against mammal seed predators either in the form of physical or chemical defenses. *Astrocaryum alatum* and *D. panamensis* both have physical defenses against seed predation in the form of hard stony endocarps that are 5 mm or 5 – 7 mm thick respectively. *Mucuna holtonii* seeds contain high concentrations of the toxic amino acid L-dopa as a chemical defense against seed predation by mammals.

For use in all subsequent experiments, I collected ripe fallen seeds and fruits during the peak fruiting seasons for each species. I collected fruits from at least 15 individual trees per species. Seeds were thereafter pooled together (by species) and a subset was chosen randomly for use in seed fate experiments. I performed experiments during the months when each respective tree species was fruiting so as to coincide with the time period when ambient abundance of each seed species on the forest floor was highest. In the following seed fate experiments, I used seeds of uniform size and mass (Table 2.1) with no evidence of insect infestation or fungal growth. To detect insect infestation, I visually inspected each seed for entrance holes indicative of infestation by Scolytid or Bruchid beetles. Any seeds with evident fungal infection or insect infestation holes were discarded.

Relative abundances of peccaries and agoutis within La Selva

To determine relative abundances of peccaries and agoutis within the La Selva forest, I used open-access camera trap data from the Tropical Ecology Assessment and Monitoring Network (TEAM, <http://www.teamnetwork.org/en/>). Between March and

June from 2006 – 2008, TEAM deployed pairs of passive infrared motion detecting camera traps (Reconyx RM45) at points throughout La Selva to monitor large terrestrial mammals (camera point densities for 2006: 1 camera point per km², 2007: 1 camera point per km², 2008: 1.25 camera points per km²). Following a standardized protocol, two cameras were deployed at each camera point within areas of previously documented high use by peccaries and agoutis (TEAM Network 2008). All photos taken by cameras were automatically time and date stamped so that events could be easily distinguished.

To collect relative mammal abundance data for 2006, 2007, and 2008, I counted the number of individuals (either peccaries or agoutis) in each picture taken by a single camera at each site in pictures taken at least five minutes apart. Cameras were equally sensitive to peccaries and agoutis. Because no bait was used to lure animals to camera points, animals moved through the camera focal area within five minutes, preventing re-counting of individual animals within single photographic events. Following this protocol, I recorded photographic captures of unique individuals at each trapping site for each year. I used these data to determine whether a seed located on the forest floor would likely be first encountered by either a peccary or an agouti, thereby affecting its fate after handling by either mammal species. I compared the relative abundances of peccaries and agoutis per site per year using a two-way ANOVA.

Times to seed depot discovery by peccaries and agoutis

To more accurately determine the likelihood of seeds on the ground being discovered first by either peccaries or agoutis, I used digital motion-detecting camera traps (Moultrie GameSpy 2.0) positioned above seed offers (approximately 2 m above

ground) to record times to seed depot discovery (methods modeled after Jansen and Ouden 2005). Because all photos were stamped with times and dates, I used this information to measure the number of days until each mammal species encountered and manipulated seeds within each of the seed depots. I compared the seed detection rates (Kaplan-Meier curves) of peccaries and agoutis for all seed species using log-rank tests.

Seed fates and seed survival over time

To monitor how peccaries and agoutis affect seed survival over time, I tracked individually numbered thread-marked seeds of *I. deltoidea*, *S. exorrhiza*, *A. alatum*, *D. panamensis*, and *M. holtonii* over periods of 36 d. For each seed species, I selected locations for seed depot pairs throughout the primary forest located in areas of high peccary and agouti use and separated by minimum distances of 400 m. At each site, I constructed two seed depots: one with a semi-permeable enclosure that excluded peccaries but allowed agouti (and small mammal) entrance and access to seeds within (caged) and another seed depot without an enclosure and a seed offer open to both peccaries and agoutis (open) (Figure 2.1). The open depot treatment contained seeds accessible by all terrestrial mammals, however, only peccaries and agoutis interacted with seeds in this study. The caged and open seed depots at each site were separated by 2 - 4 m. Seed depot sample sizes for each seed species were $N = 15$ open and $N = 15$ caged treatments except for *S. exorrhiza* where $N = 16$ for each treatment.

Each seed depot within the pair contained nine seeds of a single species arranged in a 3×3 grid; thus each seed depot pair contained a total of 18 seeds from a single seed species. Peccary enclosures consisted of $1.5 \times 1.5 \times 1$ m (L \times W \times H) galvanized metal

fencing firmly staked into the ground and supported by 1 m PVC poles at each corner. I cut one 20 × 15 cm opening on each side of the square exclosures, allowing agouti and other small mammal access to the seed offer within, but excluding peccaries (Figure 2.1). I outfitted each seed depot with digital motion-detecting camera traps (Moultrie GameSpy 2.0 and Reconyx RM45) to record the identity of seed removing animals interacting with individual seeds both within and outside exclosures. Cameras were attached to nearby trees and located approximately 2 m above ground looking down on the seed offers. Because all seeds were uniquely numbered and placed in successive order within each seed grid, I was able to record the species of terrestrial mammal that interacted with each individual seed.

To track seed fates over time and to facilitate seed relocation on the forest floor, I attached a 50 cm length of fluorescent pink nylon twine to each seed. Threads were tied through a single hole drilled in each seed. I attached a 1 × 2.5 cm uniquely-numbered aluminum tag and 10 cm of pink flagging tape to the distal end of the thread. For marked *M. holtonii* seeds, the drilled hole was filled with melted paraffin wax to prevent endosperm excavation and removal by leaf litter ants (Kuprewicz and García-Robledo 2010); based on pilot studies, leaf litter ants did not attack *I. deltoidea*, *S. exorrhiza*, *A. alatum*, or *D. panamensis* endosperm so paraffin was not used to fill the holes made in these seeds.

I checked each seed depot every two days for 36 d and surveyed the forest floor to recover seeds and strings. I recorded the number of seeds removed from the source, fates of recovered seeds (predation, dispersal, hoarding), and seed removal distances. A thread detached from a seed indicated an act of predation whereas a thread still attached to a

moved seed located above the soil surface indicated an act of seed dispersal. I could also detect seeds hoarded by agoutis as strings remained above the soil surface while the attached seed was buried below ground. I examined photographs taken by the camera traps to determine which vertebrate species removed each individual seed. I used failure time analysis (Cox Proportional Hazards model, R-Development-Core-Team 2009) to compare the relative rates of seed predation by peccaries and agoutis for each of the five seed species. I also used Wilcoxon matched pairs tests to compare the numbers of seeds of each species alive after 36 d within and outside of peccary exclosures.

Patterns of seed removal and dispersal by large terrestrial mammal frugivores

To calculate one component of seed dispersal effectiveness, seed dispersal distance, I directly measured the distances of thread-marked seeds removed from their sources after 36 d. I calculated seed removal kernels and seed dispersal kernels of all five seed species for agoutis and peccaries. Within this manuscript, I define removal as animal-mediated seed movement from the source regardless of fate (predation, dispersal, or hoarding). Alternately, dispersal refers only to the movement of intact, viable seeds moved by peccaries and agoutis. To determine if peccaries and agoutis exhibit similar or different seed movement patterns, I compared the seed removal kernels of peccaries and agoutis for each seed species using Kolmogorov-Smirnov tests. I also compared peccary and agouti seed dispersal kernels using Kolmogorov-Smirnov tests. To determine which mammal species disperses seeds farthest from the source, I used Wilcoxon rank sum tests to compare the median distances of seeds dispersed by peccaries and agoutis for each seed species.

RESULTS

Relative abundances of peccaries and agoutis within La Selva

I surveyed photographs from a total of 16 camera sites in 2006, 16 sites in 2007, and 20 sites in 2008. Abundances of both peccaries and agoutis remained constant from 2006 through 2008 in La Selva. However, within each year peccaries were more abundant than agoutis, as indicated by two-way ANOVA (Table 2.2, Figure 2.2).

Times to seed depot discovery by peccaries and agoutis

I measured the times until peccaries and agoutis discovered seeds at 30 seed depots for each seed species (except for *S. exorrhiza* which had a total of 32 depots). For all seed species, I found that peccaries encountered seed depots significantly more rapidly than agoutis (Figure 2.3). After a period of 36 d, peccaries discovered *I. deltoidea* seeds more quickly than agoutis ($\chi^2 = 10$, $df = 1$, $P < 0.002$; Figure 2.3a). Peccaries also were first to find *S. exorrhiza* seeds ($\chi^2 = 25.9$, $df = 1$, $P < 0.001$; Figure 2.3b), *A. alatum* seeds ($\chi^2 = 5$, $df = 1$, $P < 0.03$; Figure 2.3c), *D. panamensis* fruits ($\chi^2 = 9.2$, $df = 1$, $P < 0.0024$; Figure 2.3d), and *M. holtonii* seeds ($\chi^2 = 26.6$, $df = 1$, $P < 0.001$; Figure 2.3e). At the end of the study period, peccaries had encountered 93% of *I. deltoidea* seed depots, 100% of *S. exorrhiza* depots, 87% of *A. alatum* depots, 93% of *D. panamensis* depots, and 100% of *M. holtonii* depots whereas agoutis had encountered 60%, 13%, 60%, 40%, and 7% of these depots respectively (Figure 2.3).

Seed fates and seed survival over time

Based on photographs taken by cameras at seed depots, I found that, peccaries and agoutis were the only terrestrial mammals that interacted with thread-marked seeds. Although pacas (*Cuniculus paca*, Rodentia), spiny rats (*Proechimys semispinosus*, Rodentia), squirrels (*Sciurus* spp., Rodentia), and armadillos (*Dasypus novemcinctus*, Cingulata) were observed visiting some seed depots and inspecting seeds, none of these animals consumed or moved thread-marked seeds.

I recovered most seeds and strings that were moved or handled by seed predators. I relocated 139 of 145 displaced *I. deltoidea* seeds (99% recovered), 148 of 151 removed *S. exorrhiza* seeds (98 % recovered), 86 of 88 *A. alatum* seeds (98% recovered), 126 of 131 *D. panamensis* fruits (96% recovered) and 135 of 135 *M. holtonii* seeds (100% recovered).

For all seed species, relative rates of seed predation in open depots (exposed to both peccaries and agoutis) were significantly faster than predation rates of seeds within peccary enclosures. Species-specific pairwise comparisons of caged and open seed treatments revealed significant differences in the relative rates of seed predation within and outside of peccary enclosures for *I. deltoidea* ($\chi^2 = 179.2$, $df = 1$, $P < 0.001$), *S. exorrhiza* ($\chi^2 = 275.5$, $df = 1$, $P < 0.001$), *A. alatum* ($\chi^2 = 5.3$, $df = 1$, $P < 0.03$), *D. panamensis* ($\chi^2 = 42.6$, $df = 1$, $P < 0.001$), and *M. holtonii* (no statistical comparison required to test evident difference in predation rates) (Figure 2.4).

For all seed species except *A. alatum*, median numbers of seeds alive after 36 d were higher for seeds protected from peccaries compared to seeds exposed to peccaries. Comparisons of caged and open seed depots found significant differences in the median

numbers of seeds surviving to day 36 for *I. deltoidea* ($V = 119$, $P < 0.001$), *S. exorrhiza* ($V = 120$, $P < 0.001$), *D. panamensis* ($V = 75$, $P < 0.005$), and *M. holtonii* ($V = 120$, $P < 0.001$). There was no significant difference in the median number of *A. alatum* seeds alive after 36 d for seeds protected from and exposed to peccaries ($V = 15$, $P = 0.40$).

Patterns of seed removal and dispersal by large terrestrial mammal frugivores

Within 36 d, peccaries had removed 126 *I. deltoidea* seeds, 136 *S. exorrhiza* seeds, 25 *A. alatum* seeds, and 102 *D. panamensis* fruits. Agoutis removed 28 *I. deltoidea* seeds, 20 *S. exorrhiza* seeds, 79 *A. alatum* seeds, and 53 *D. panamensis* fruits. Neither peccaries nor agoutis moved *M. holtonii* seeds from their sources. Peccary and agouti seed removal kernels for *I. deltoidea* did not differ significantly in their distributions ($D = 0.23$, $P = 0.16$) whereas comparisons of peccary and agouti seed removal kernels showed significant differences for *S. exorrhiza* ($D = 0.34$, $P < 0.02$), *A. alatum* ($D = 0.42$, $P < 0.003$), and *D. panamensis* ($D = 0.33$, $P < 0.001$) (Figure 2.5).

Overall, dispersal and hoarding of intact seeds was infrequent within this study. At the end of the study period, peccaries had dispersed 16 *I. deltoidea* seeds, 6 *S. exorrhiza* seeds, 24 *A. alatum*, and 29 *D. panamensis* fruits. Agoutis only dispersed 3 *I. deltoidea* seeds, 1 *S. exorrhiza* seed, 18 *A. alatum* seeds, and 8 *D. panamensis* fruits. No *M. holtonii* seeds were dispersed by either mammal. Comparisons of seed dispersal kernels produced by peccaries and agoutis showed that dispersal distance distributions were not significantly different for any of the seed species (*I. deltoidea*: $D = 0.31$, $P = 0.92$; *S. exorrhiza*: $D = 0.83$, $P = 0.71$; *A. alatum*: $D = 0.17$, $P = 0.91$; *D. panamensis*: $D = 0.41$, $P = 0.25$) (Figure 2.6). Median distances of seeds dispersed by peccaries and

agoutis also did not differ for *I. deltoidea* ($W = 25$, $P = 0.96$), *S. exorrhiza* ($W = 5$, $P = 0.57$), *A. alatum* ($W = 217$, $P = 0.85$), and *D. panamensis* ($W = 78.5$, $P = 0.17$).

DISCUSSION

Collared peccaries and Central American agoutis are the most abundant terrestrial mammal species at La Selva (TEAM Network, <http://www.teamnetwork.org/en/>). Both of these mammals are generalist frugivores that have likely proliferated because of local large felid rarity (release from predation pressure), high abundance of fruit and seeds available year-round, and protection from human hunters. Because frugivore abundances remained at constant levels throughout this study, their populations may be operating at or near carrying capacity within this forest. When comparing the relative abundances of peccaries and agoutis within La Selva, peccaries were up to six times more abundant than agoutis from 2006 – 2008. Peccaries produce larger litters more frequently than agoutis, likely contributing to abundance disparities between these mammal species (Reid 1997, Smythe 1978).

High abundances of peccaries relative to agoutis resulted in all experimental seeds being encountered first by peccaries in the field. The probability of a seed on the forest floor being found and handled by a peccary is higher than the likelihood of a seed being encountered by an agouti as indicated by shorter times to seed depot discovery by peccaries. The respective foraging strategies of these mammal species also likely affect seed encounter rates. Collared peccaries forage in large groups (2 – 50 individuals) (Castellanos 1983, Judas and Henry 1999) and these aggregates cover large foraging areas in the forest enabling peccaries to encounter and consume many recently fallen

seeds. I have observed groups of up to 30 individuals in La Selva, however most groups contained 3 – 15 individuals (pers. obs.). In contrast, agoutis are solitary foragers that sometimes travel in foraging pairs within their territories (Smythe 1978, pers. obs.). Additionally, the home ranges of these two mammal species differ greatly and probably contribute to differences in seed encounter rates. Collared peccaries forage within large home ranges varying from 38 ha (Castellanos 1985) to 685 ha (Taber *et al.* 1994, Carillo *et al.* 2002) whereas Central American agoutis have much smaller home ranges of 1 – 4 ha (Hallwachs 1986, Smythe 1978). Silvius and Fragoso (2003) measured directly red-rumped agouti (*Dasyprocta leporina*, Linnaeus 1758; a congener of the Central American agouti) home ranges that varied from 2.9 – 8.5 ha. With agouti home ranges and foraging areas being much smaller than those of peccaries, a seed located on the forest floor is more likely to fall within an area traversed by many peccaries rather than a small territory containing an agouti pair.

Most seeds in this study were encountered by peccaries and subsequently destroyed through seed predation. Ultimate seed fate, however, was contingent on the presence or quality of seed defenses. Seed species lacking apparent anti-predation defenses (*I deltoidea*, *S. exorrhiza*), one seed species with structural defenses (*D. panamensis*), and chemically-defended seeds (*M. holtonii*) suffered from intense predation by peccaries within 36 d. When protected from peccaries and exposed only to agoutis, these seed species had high percentages of seed survival (76% - 100%). This result indicates that agoutis do not kill the majority of seeds they encounter within 36 d and peccaries predominantly negatively affect seed survival through predation.

Seeds lacking defenses suffered high mortality from peccary predation due to high encounter rates with these abundant, group-foraging ungulates. *Iriartea deltoidea* (= *I. ventricosa*) and *S. exorrhiza* seeds are attractive food sources for terrestrial granivores and were readily consumed by collared peccaries that could easily masticate these nutritious seeds (pers. obs.). My results, however, contradict earlier assertions made by Kiltie (1982), based on field observations and feeding trials in Peru, that collared peccaries could not crush and consume seeds of these two palm species. Although young peccaries could not crush *I. deltoidea* or *S. exorrhiza* seeds in the field (pers. obs.), adults readily consumed almost 100% of these seeds after 36 d. Perhaps *I. deltoidea* and *S. exorrhiza* seeds in Manú are harder or possess thicker seed coats than seeds found in La Selva, preventing access to this food source by collared peccaries in that location. Reciprocal selection pressures (on seed defense structures and mammal bite force physiology) may be playing a role in the seed predation success or failure of large terrestrial granivores.

Contrary to my expectations, peccaries destroyed a higher proportion of physically-defended *D. panamensis* seeds than agoutis. These seeds are protected by a thick (5 – 7 mm), stony endocarp but peccaries were able to open *D. panamensis* fruits and consume seeds. When *D. panamensis* fruits are fresh, the suture encircling the endocarp is tightly sealed and peccaries cannot break them, but as fruits age, this suture weakens, the fruit pod (endocarp) dehisces to allow radicle and plumule emergence, and peccaries can open fruits along the seam to extract seeds within (Enders 1935, pers. obs.). Physical defense against seed predation for *D. panamensis* is ephemeral as seeds are protected from peccary predation only when freshly dropped from parent trees.

Agoutis, however, can open *D. panamensis* fruits at any stage of ripeness by gnawing through the endocarp (pers. obs.).

Peccaries possess complex stomachs capable of foregut fermentation, allowing them to consume toxic *M. holtonii* seeds that agoutis cannot process (Daxenbichler *et al.* 1972, Carl & Brown 1983, Olmo 1993, Elston *et al.* 2005, Nogueira 2005, Kuprewicz and García-Robledo 2010). All *M. holtonii* seeds within peccary exclosures survived to the end of the study, indicating that agoutis did not consume any seeds of this species, whereas peccaries consumed and destroyed 100% of the seeds they could access. The chemical defenses of *M. holtonii* prevent seed predation by agoutis, but these defenses do not offer complete protection from terrestrial mammal seed predation because peccaries can digest toxic seeds.

The only seed species immune to predation by peccaries was *A. alatum*. Peccaries did not consume any seeds of this physically-defended species; all predation was by agoutis. The bite force provided by collared peccary jaws was not strong enough to break through the thick (5 mm) endocarp that *A. alatum* seeds possess. In feeding trials involving 30 wild peccaries in La Selva (E. K. Kuprewicz, unpubl. data), all individuals made vigorous attempts to crush *A. alatum* seeds, but none could break through the thick endocarp. Peccaries abandoned seeds after approximately 10 – 120 s. Due to their inability to consume *A. alatum* seeds, peccaries may positively affect this tree species as incidental seed dispersers that expectorate seeds while foraging and thus move them short distances from beneath parent trees (Beck 2005). In other studies in Peru and Panama, collared peccaries have been observed cracking endocarps and consuming seeds of *Astrocaryum murumuru* (= *macrocalyx*) and *A. standleyanum*

(Smythe 1978, Kiltie 1982). These *Astrocaryum* species likely possess thinner endocarps than *A. alatum* present within La Selva, allowing *A. murumuru* and *A. standleyanum* to be exploited by collared peccaries.

Alternately, all *A. alatum* seed predation within this study was due to agoutis. After 36 d, *A. alatum* seed survival within and outside peccary exclosures did not differ indicating equivalent discovery and handling of seeds by agoutis in both treatments. Physical defenses did not deter seed predation by agoutis that gnawed through to *A. alatum* seeds by repeatedly rasping the endocarp with their sharp incisors (pers. obs.).

In this study, seed dispersal by mammals and hoarding by agoutis was low. I only observed 10 total hoarding events in the course of this study (1 *S. exorrhiza*, 5 *A. alatum*, and 4 *D. panamensis*). The low frequency of hoarding within La Selva may be attributable to the lack of seasonality within this wet forest (no dry season) and resultant elevated levels of year-round fruit abundance. Agoutis may not hoard often because there is no seasonal fruit and seed scarcity here. Also, seeds may not be found and hoarded by agoutis because peccaries encounter and destroy most seeds before agoutis can locate them. Although scatter-hoarding of seeds by agoutis enhances seed survival and germination (*e.g.* Forget 1993, Forget 1994, Brewer and Rejmánek 1999, Kuprewicz Chapter 4 this dissertation), this phenomenon likely does not contribute significantly to tree recruitment and propagation within La Selva.

Peccaries and agoutis differed in their patterns of seed movement for most seed species. Peccaries and agoutis displayed different seed removal distance distributions for *S. exorrhiza*, *A. alatum*, and *D. panamensis*, but not for *I. deltoidea* or *M. holtonii* seeds. Overall, peccaries moved most seeds within 10 m of the source with a few long distance

dispersal events (> 30 m). Agoutis also moved seeds mostly short distances, but long distance dispersal by this species was rare. Contrary to my previously posited hypotheses pertaining to seed dispersal distances, peccaries and agoutis exhibited no differences in seed dispersal kernels or the distances they dispersed intact, viable seeds. These mammals are equally effective seed dispersers with regard to distance, though agoutis may provide more effective qualitative dispersal by hoarding some dispersed seeds below the soil surface, effectively hiding seeds from vertebrate and invertebrate seed predators (Fragoso 1997, Silvius 1999, Kuprewicz Chapter 4 in this dissertation).

Seeds with no defenses or chemical defenses against mammal seed predation face high predation pressure within La Selva because peccaries are locally abundant. In contrast, peccary populations are greatly reduced or locally extinct in the hunted, fragmented areas surrounding La Selva. Extirpation of granivorous megafauna has resulted in proliferation of trees usually regulated by seed predation (Silman *et al.* 2003, DeMattia 2004). Loss of peccaries from La Selva would have profound effects on forest community structure and composition, with an expected increase in seed survival, germination, and seedling recruitment of *I. deltoidea*, *S. exorrhiza*, *D. panamensis*, and *M. holtonii*. Additionally, with the loss of peccaries, agouti populations may increase due to release from competition (Smythe 1978, Smythe 1986). An increase in agouti abundance within La Selva might result in a concomitant decline of physically-defended plant seeds and resultant seedlings under more intense agouti-mediated seed predation.

In conclusion, physical and chemical defenses do not protect seeds from all mammal seed predators. Extrinsic factors and intrinsic traits of mammal granivores and the seed species they consume interact to produce variable seed fate outcomes. La Selva

is a unique forest with high peccary abundances and this has far-reaching implications for seed survival and tree propagation. Peccaries encounter seeds on the forest floor quickly and, if seeds are not protected by tough endocarps, consume and destroy a majority of the seed crop. Chemically-defended seeds are effectively protected from agoutis and other granivorous rodents, but peccaries are immune to seed toxicity. Peccaries may disperse hard-walled seeds incidentally, potentially leading to a proliferation of trees that produce seeds encased in (non-ephemeral) hard endocarps. Although peccaries and agoutis differ in home ranges and foraging techniques, they produce similar seed shadows at La Selva. By analyzing seed encounter rates and seed handling strategies of mammal seed predators and how they interact with seeds, we can determine how mammal and plant communities may change with natural or anthropogenic disturbance.

Table 2.1. Seed and fruit characteristics of all plant species used in seed-tracking experiments. Sample sizes for masses: *Astrocaryum alatum* $N = 104$, *Iriarteia deltoidea* $N = 50$, *Socratea exorrhiza* $N = 69$, *Dipteryx panamensis* $N = 14$.

Diaspore	Family	Defense	Mass (g) Mean \pm 1 SD	Dimensions
<i>Iriarteia deltoidea</i>	Arecaceae	None	2.9 \pm 0.9	diameter = 2-2.8 cm
<i>Socratea exorrhiza</i>	Arecaceae	None	3.6 \pm 0.6	length = 2.5-3.5 cm, diameter = 1.5-2 cm
<i>Astrocaryum alatum</i>	Arecaceae	Physical (hard endocarp)	25.1 \pm 4.5	length = 6 cm, width = 4 cm
<i>Dipteryx panamensis</i>	Fabaceae	Physical (hard endocarp)	25.2 \pm 4.4	length = 6 cm, width = 3 cm
<i>Mucuna holtonii</i>	Fabaceae	Chemical (toxic L-dopa)	5.36 \pm 0.7	diameter = 2-3 cm, thickness = 0.8 cm

Table 2.2. Two-way ANOVA comparing La Selva peccary and agouti abundances within and across three consecutive years (2006, 2007, 2008).

Source of variation	<i>df</i>	Sum of squares	<i>F</i>	<i>P</i>
Year	1	1413	1.74	0.19
Mammal	1	8354	10.31	> 0.002
Year × Mammal	1	259	0.32	0.57
Residuals	96	77757		

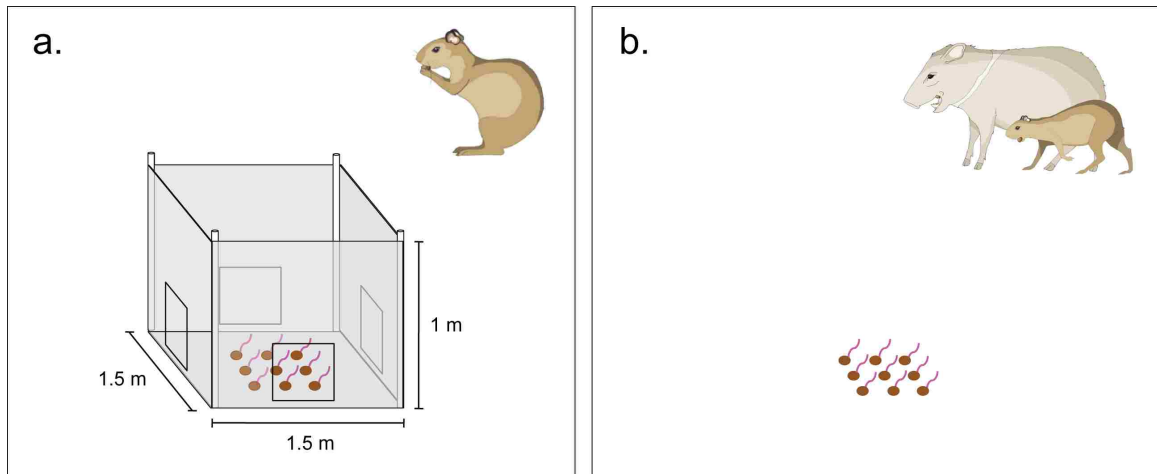


Figure 2.1. Diagram depicting seed depot pairs used in seed fate experiments. One depot in the pair contained an offer of nine thread-marked seeds housed within a peccary enclosure that only allowed agouti entrance and access to the seeds (a). The other depot, located 2 – 4 m from the enclosure, consisted of an identical marked seed offer, but was open to allow access to the seeds by peccaries and agoutis (b).

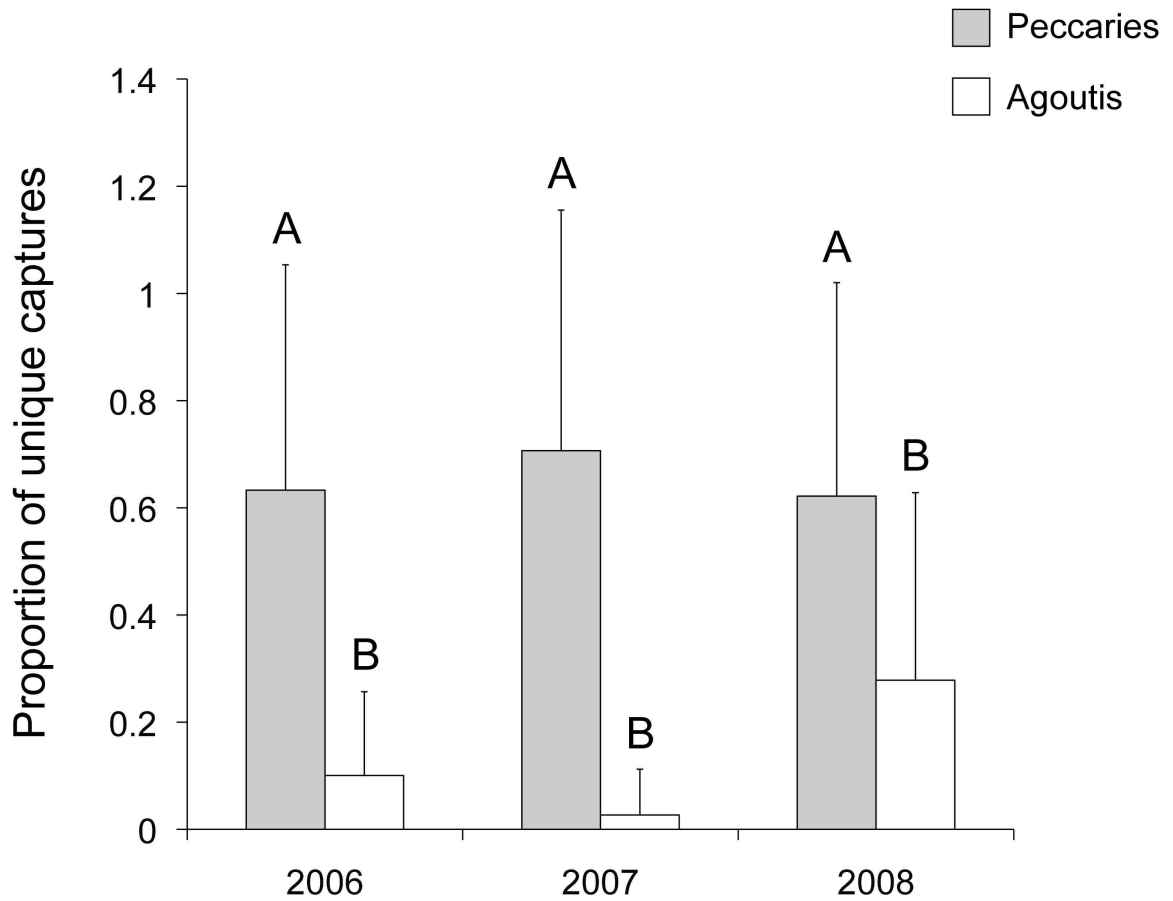


Figure 2.2. Mean proportion of unique photo captures (+ 1 SD) of collared peccaries (*Pecari tajacu*, gray bars) and Central American agoutis (*Dasyprocta punctata*, white bars) per site per year. Letters group similar categories. Number of sites monitored in 2006 = 16, 2007 = 16, 2008 = 20.

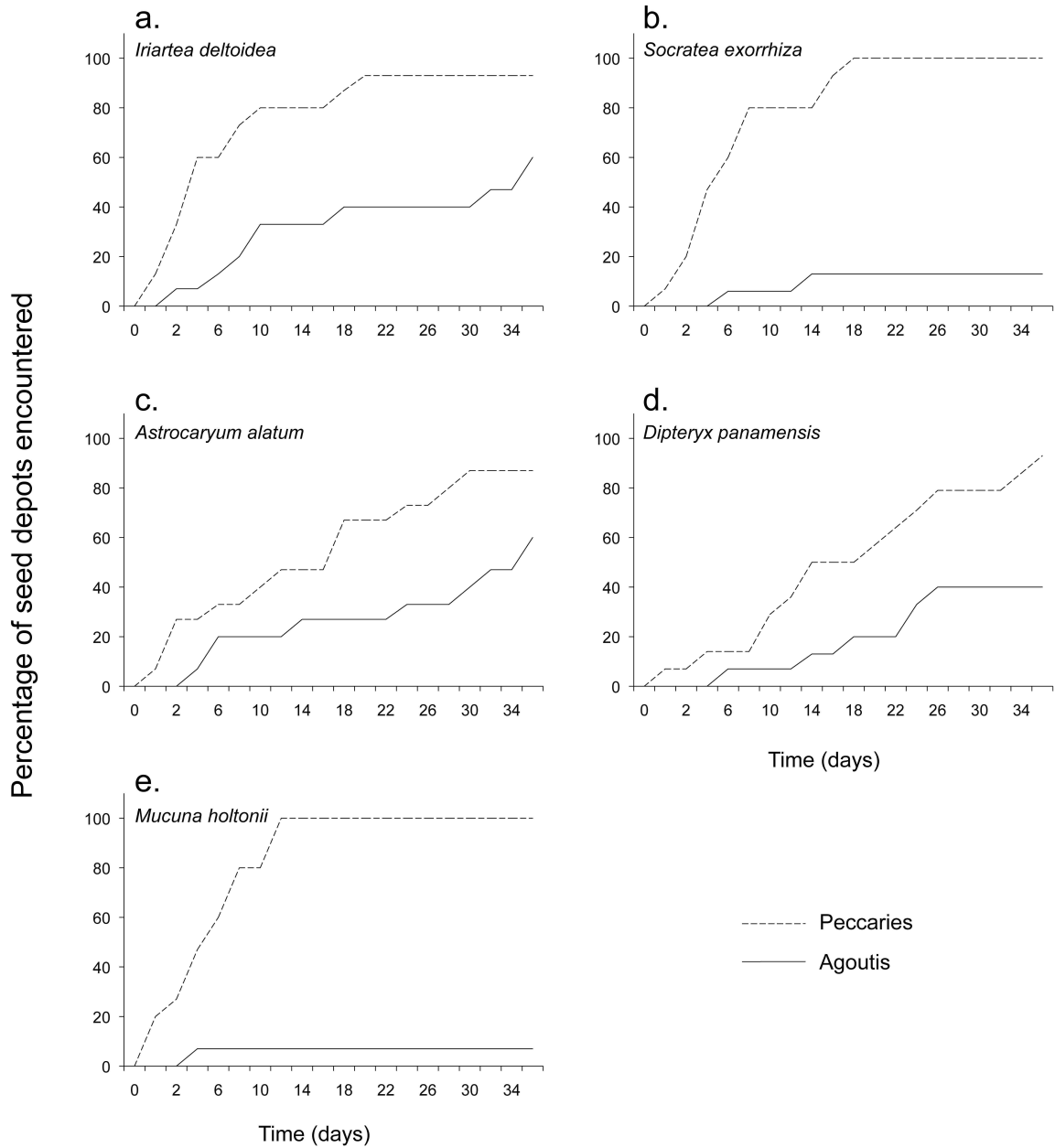


Figure 2.3. Seed depot discovery by terrestrial frugivores. Cumulative percentage of seeds of *I. deltoidea* (a), *S. exorrhiza* (b), *A. alatum* (c), fruits of *D. panamensis* (d), and seeds of *M. holtonii* (e) encountered by peccaries and agoutis over 36 d.

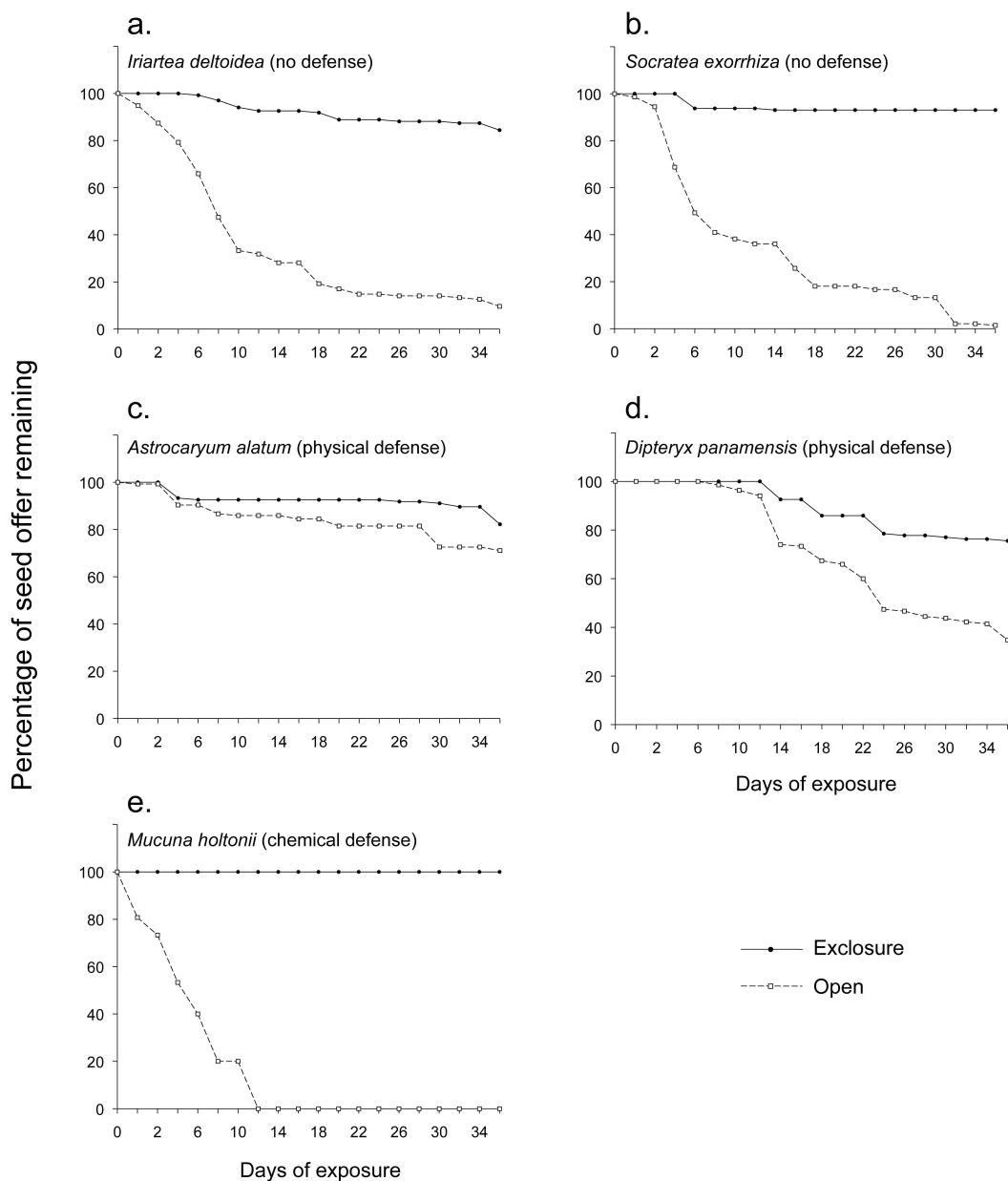


Figure 2.4. Mean percent per depot of surviving seeds of *I. deltoidea* (a), *S. exorrhiza* (b), *A. alatum* (c), fruits of *D. panamensis* (d), and seeds of *M. holtonii* (e) inside (exclusion, filled circle solid line) and outside (open, open square dashed line) semi-permeable mammal enclosures. Seeds within enclosures were exposed to agoutis only and seeds outside of enclosures were available to all terrestrial mammals (peccaries and agoutis). $N = 30$ seed depots (15 exclusion, 15 open) for all plant species except *S. exorrhiza*, $N = 32$ depots (16 exclusion, 16 open).

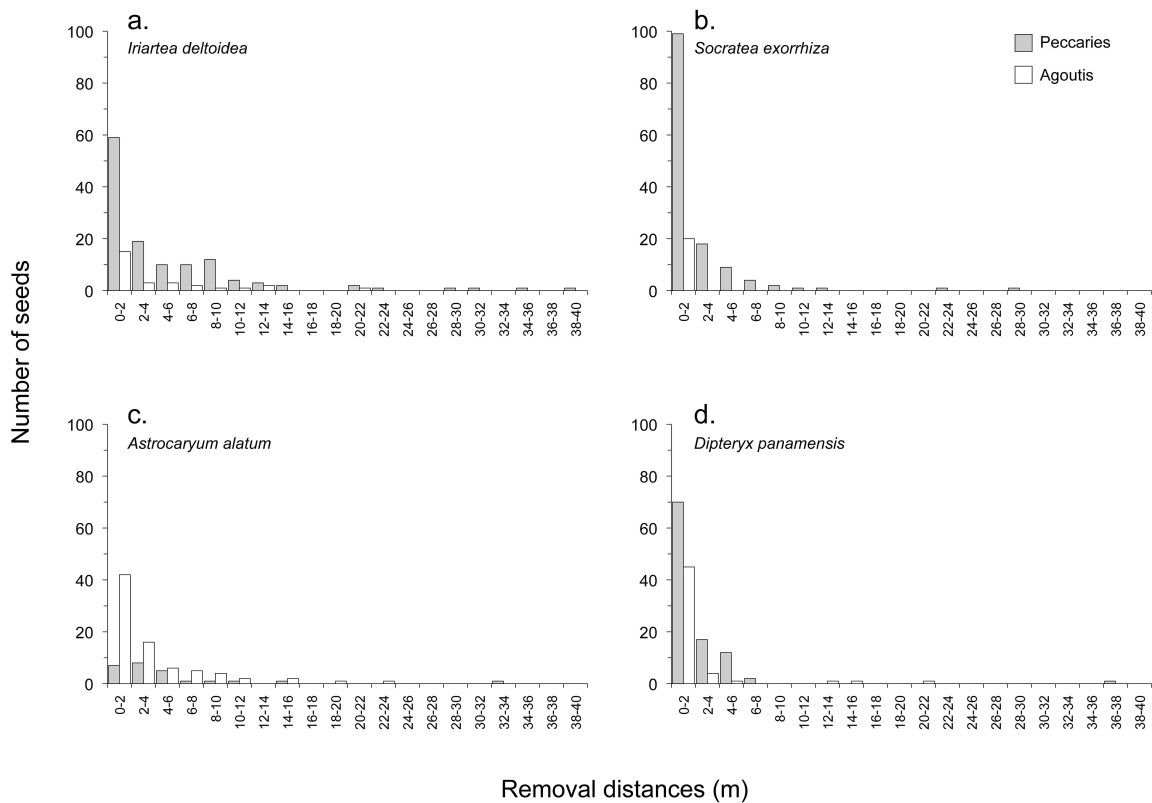


Figure 2.5. Frequency distributions of *I. deltoidea* (a), *S. exorrhiza* (b), *A. alatum* (c), and *D. panamensis* (d) seed removal distances by peccaries (gray bars) and agoutis (white bars).

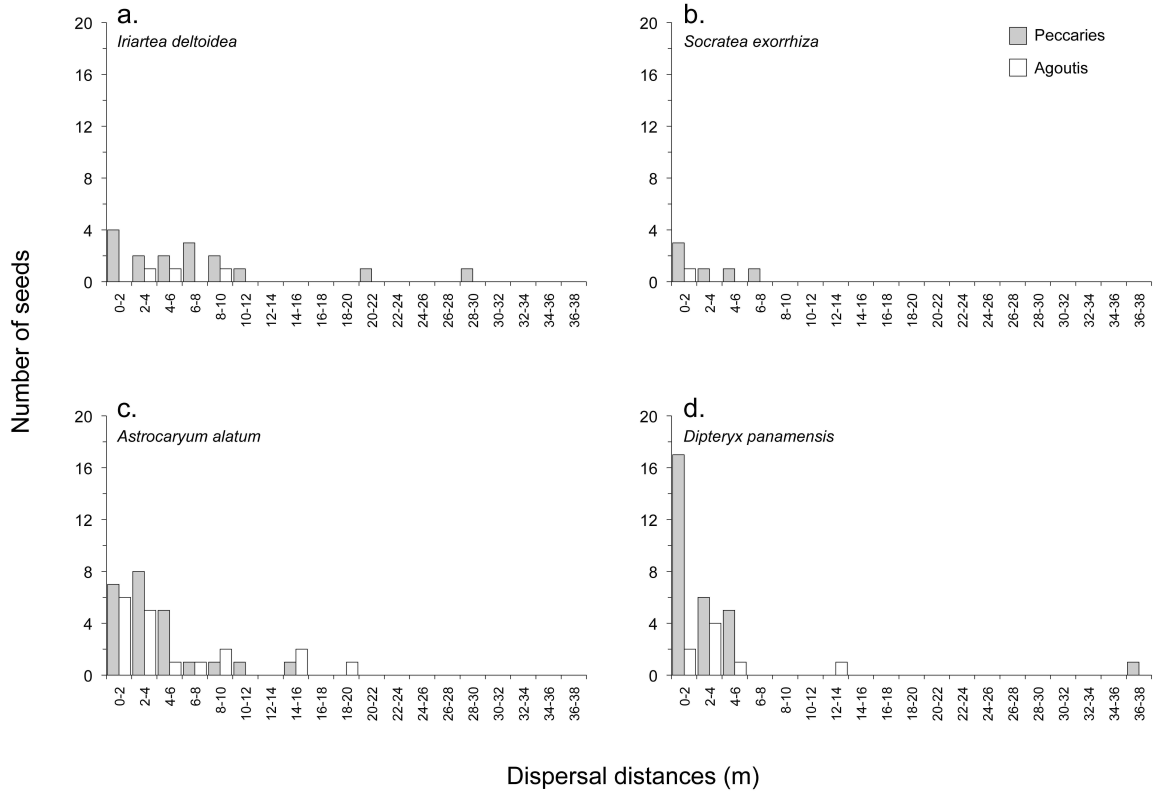


Figure 2.6. Frequency distributions of *I. deltoidea* (a), *S. exorrhiza* (b), *A. alatum* (c), and *D. panamensis* (d) seed dispersal distances by peccaries (gray bars) and agoutis (white bars).

CHAPTER 3

Mammal and insect predation of chemically and structurally defended *Mucuna holtonii* (Fabaceae) seeds in a Costa Rican rain forest¹

SUMMARY

To prevent seed losses from predation, plants have developed protective strategies. Seeds may utilize chemical or structural defenses to deter predators. *Mucuna holtonii* (Fabaceae) has large seeds containing a toxic amino acid, L-dopa, and covered with a hard seed coat. This study assessed the effectiveness of chemical and mechanical seed defenses against vertebrate and invertebrate seed predators within La Selva Biological Station, Costa Rica. Pre-dispersal insect and fungus attack of *M. holtonii* seeds was low (95.7% of 1493 seeds were undamaged). Camera traps monitoring 90 marked *M. holtonii* seeds showed that collared peccaries (*Pecari tajacu*) consumed 98.6% of 69 removed seeds over 16 d. Field experiments involving 100 seeds with intact and 100 with opened seed coats found that only opened seeds had endosperm removed by *Sericomyrmex amabilis* ants (0.5%-100% of endosperm removed). Shade-house experiments showed that seeds with high amounts of endosperm removed by ants resulted in low germination success and low seedling biomass production. Although *M. holtonii* seeds are rich in L-dopa, this compound is not an effective chemical defense against mammals that possess foregut fermentation. The seed coat of *M. holtonii* is an effective structural defense against invertebrate seed predators, preventing endosperm removal and enhancing seedling survival.

¹ Coauthor: Carlos García-Robledo

BACKGROUND

Seed predation by vertebrates and invertebrates is a major factor limiting the colonization, establishment and growth of plant populations (Janzen 1971a, b, Howe and Smallwood 1982, Schupp 1988, Forget 1993, Forget *et al.* 1999). Seed-eating animals may attack seeds during fruit development while seeds and fruits are still attached to parent plants; this is termed pre-dispersal seed predation. Plants can also suffer from post-dispersal seed predation when ripe seeds are consumed after their release from the parent plant. To prevent high seed losses and a consequent reduction in fitness, many plants have developed protective strategies against seed predators. These strategies include physical protection, such as spines or hard fruit endocarps that serve as barriers to predation, and chemical defenses that render seeds toxic or inedible to seed-eating animals (Janzen 1971b, Kiltie 1982, Bell 1984, Dirzo and Dominguez 1986, Bodmer 1991).

In tropical rain forests, seeds of several genera in the Fabaceae are chemically defended (Janzen *et al.* 1986, McKenna and McKenna 2006). One classic example of a legume with chemically defended fruits is *Mucuna*, a pantropical genus of lianas comprising approximately 100 species (Gentry 1996). *Mucuna* seeds are highly toxic as they contain high concentrations of L-dopa (3,4-dihydroxyphenylalanine) within seed embryos and cotyledons (Daxenbichler *et al.* 1972, Modi *et al.* 2008). This amino acid has pronounced deleterious effects on many insects (Rehr *et al.* 1973), mammals (Emenalom *et al.* 2004) and birds (Harms *et al.* 1961) that consume these seeds. Although *Mucuna* seeds are highly toxic, they contain large amounts of protein (Daxenbichler *et al.* 1972, Harms *et al.* 1961, Vadivel and Janardhanan 2000).

Therefore, the seeds of *Mucuna* may serve as a nutritious food resource to granivores that can overcome the seeds' chemical anti-predation defenses (Udedibie and Carlini 1998b, a, Emenalom *et al.* 2004).

The chemical properties of one species of *Mucuna*, *M. holtonii* (Kuntze) Moldenke, (syn. *M. andreana* Micheli) have been particularly well documented ((Daxenbichler *et al.* 1972), (Rehr *et al.* 1973)). The concentration of L-dopa within the embryos and cotyledons of *M. holtonii* seeds is one of the highest recorded for this genus (13.8% in immature seeds) (Daxenbichler *et al.* 1972). Although *M. holtonii* seeds are highly toxic, I have observed terrestrial mammals investigating and manipulating these seeds at La Selva Biological Station, a tropical rain forest in Costa Rica.

Previous studies have focused only on the function of *M. holtonii* chemical defenses against seed predators (Rehr *et al.* 1973). However, little is known about the potential role of seed structures, such as the seed coat, that may act as physical deterrents to seed predation (Janzen 1977a). It is possible that the thin (0.6 mm thick), coriaceous seed coat that surrounds *M. holtonii* seeds prevents insect attack (*e.g.* by bruchid beetles, (Janzen 1977a). Therefore, in this research we also explored the role of the seed coat as a mechanical barrier against invertebrate seed predation.

The overarching hypotheses that we tested within this study pertained to how seed defenses prevent seed predation. The high toxicity of *M. holtonii* seeds should prevent predation by vertebrate seed predators that cannot overcome the seeds' L-dopa defenses. Additionally, the physical protection proved by the coriaceous seed coat of *M. holtonii* seeds should act as an effective barrier to infestation by small invertebrate seed predators. To assess the effectiveness of chemical and mechanical defenses of *M. holtonii* seeds

against vertebrate and invertebrate seed predators respectively, the main objectives of our research were: (1) to assess levels of pre-dispersal insect infestation and fungal attack on ripe *M. holtonii* seeds, (2) to identify seed-removing mammals and the fates of the *M. holtonii* seeds they remove and (3) to determine if the seed coat affects insect infestation and subsequent endosperm removal. Finally, we explored the effects of endosperm removal on the germination and seedling growth from seeds partially consumed by invertebrate seed predators.

METHODS

Study site

This study was conducted from December 2007 to July 2008 at La Selva Biological Station (henceforth La Selva) located in Puerto Viejo Sarapiquí, Heredia, Costa Rica (10°26' N, 83°59' W). La Selva is a 1600-ha tropical lowland wet forest reserve comprising old-growth forest, secondary growth, swamps and tree plantations. Rainfall at this aseasonal site averages approximately 4000 mm y⁻¹ (McDade *et al.* 1994). Due to hunting and habitat fragmentation in the areas surrounding La Selva, some large-mammal species are locally extinct within this forest (*e.g.* white-lipped peccary, *Tayassu pecari*). We conducted the following study throughout the secondary forest of La Selva, corresponding to the habitat where *M. holtonii* is most abundant.

Study species

Mucuna holtonii is a tropical rain-forest liana that ranges from Chiapas, Mexico to Colombia and grows throughout secondary forest environments and disturbed areas

(Woodson Jr. and Scherry 1980). The pendulous infructescences of *M. holtonii* comprise 1-9 flat, oblong legumes (length = 14-25 cm, width = 5 cm). Each *M. holtonii* fruit pod contains 1-6 black discoid seeds (diameter = 2-3 cm, thickness = 0.8 cm) (Woodson Jr. and Scherry 1980). Upon maturation, *M. holtonii* legumes dehisce and the mature seeds fall to the forest floor where they may be encountered by terrestrial vertebrates and invertebrates.

Pre-dispersal insect infestation of Mucuna holtonii seeds

To assess how invertebrate seed predators affect *M. holtonii* seeds prior to fruit dehiscence and seed fall, we collected seeds from mature fruits and inspected each seed for invertebrate damage (*e.g.* entrance holes, portions of eaten endosperm) and fungal infection. Mature, undehisced fruits were collected directly from randomly chosen lianas to ensure that we were measuring only pre-dispersal seed damage. We collected fruits from 10 locations throughout the secondary forest habitat of La Selva. Collecting locations were separated by a minimum distance of 200 m to ensure independence. We performed an arcsine transformation on the data and compared the proportions of undamaged seeds and seeds infested by invertebrates and fungi for each location using a one-way ANOVA.

Vertebrate seed removal and seed fates of Mucuna holtonii

To determine if terrestrial animals remove and potentially consume or disperse *M. holtonii* seeds, we used digital motion-detecting camera traps (Moultrie GameSpy 200) to

monitor vertebrate removal of marked seeds. Fifteen seed depots separated by a minimum distance of 200 m were placed throughout the secondary forest of La Selva.

Each depot consisted of six fresh *M. holtonii* seeds that were individually marked with a 50-cm length of fluorescent pink nylon twine. Threads were tied through a single hole drilled in each seed. This hole was subsequently filled with melted paraffin wax to prevent invertebrate access to the seed endosperm. The distal end of each thread was attached to a 1 × 2.5-cm numbered aluminum tag to identify each individual seed and 10 cm of pink flagging tape to facilitate subsequent seed and string relocation on the forest floor. All seeds used in experiments were of equivalent weights (mean ± 1 SD = 5.4 ± 0.7 g).

Cameras were positioned 1.5 m from each group of seeds at each seed depot. From this distance, our cameras could detect small mammals (*e.g.* *Heteromys desmarestianus* Gray, 1968; *Proechimys semispinosus* Tomes, 1860) and larger mammals (*e.g.* *Dasyprocta punctata* Gray, 1842; *Nasua narica* Linnaeus, 1766; *Pecari tajacu* Linnaeus, 1758) visiting the seeds offered (García-Robledo and Kuprewicz 2009). We checked each depot daily and recorded the number of seeds removed, removal distances from each source and seed fates after removal (*i.e.* dispersed or consumed by vertebrates). Seeds remained in the field for 16 d (over 80% of all seeds were removed within this time period). We examined photographs taken by the motion-detecting camera traps to determine which vertebrate species removed each *M. holtonii* seed and we used relocated seeds and strings to determine the fates of the seeds each animal moved.

Seed coat as a structural defense against invertebrates in Mucuna holtonii

To ascertain if the seed coat plays a role in protecting *M. holtonii* seeds from invertebrate seed predators, we performed the following experiment. We randomly selected 10 locations spaced at least 200 m from each other throughout the secondary forest of La Selva where *M. holtonii* lianas were found. At each location, we placed seeds in two wire mesh boxes (L = 15 cm, W = 15 cm, H = 2 cm, mesh size = 2 × 2 cm) that allowed access to the seeds by invertebrates (and incidentally fungi and pathogens) but prevented vertebrate access.

We placed five *M. holtonii* seeds with intact seed coats (intact seeds) into each mesh box along with five seeds from which we removed two small (diameter = 4 mm) slices of the seed coat, one slice on the upper face and the other on the lower face of the seed (opened seeds). We recorded any invertebrate observed consuming *M. holtonii* seed endosperm after 24 h.

These seeds remained in the field for 16 d, whereupon they were brought to a laboratory to measure the amount of endosperm removed by invertebrates. To determine total seed volumes, we closed any hole in the seed coat with Parafilm® and placed seeds in a 50-ml beaker filled with water. We measured the volume of water displaced using pipettes with an accuracy of 0.01 ml. For each seed, we subsequently measured the volume of endosperm removed by invertebrates by filling the excavated hole(s) with water and recording the volume of water required to fill the seed. From these two values (intact seed volume and excavated endosperm volume), we calculated the percent of endosperm removed by invertebrates from each seed. For each depot, we compared the

mean percent of endosperm removed by invertebrates for intact seeds and opened seeds using a match-paired t-test.

Effects of endosperm removal by invertebrates on Mucuna holtonii seed germination and growth

To determine how endosperm removal affects the germination success of *M. holtonii* seeds, we selected seeds with similar original masses (mean = 5.36 g, SD = 0.71 g, N = 50) but with different amounts of endosperm removed by invertebrates in the field (range of endosperm removed = 0%-100%). The volume of endosperm removed was recorded for each seed. Seeds were placed in independent germination bags (volume = 1178 cm³) filled with soil from the secondary forest and monitored for 45 d under natural light and water conditions in a shade house. We used logistic regression to explore the effect of endosperm removal on seed germination.

To determine the effect of invertebrate endosperm removal on seedling growth of *M. holtonii*, we measured root and shoot biomass production in seeds with different amounts of endosperm removed by invertebrates. Seeds were harvested 15 d after radicle and plumule emergence, whereupon we removed the root and shoot from the remaining cotyledons. We subsequently dried the roots and shoots produced by each seed in a 60° C drying oven for 48 h. We measured the dry weights of the roots and shoots using an analytical balance with an accuracy of ± 0.01 g. The effects of endosperm removal on root and shoot biomass production were explored with linear regressions. Data were transformed using the Box-Cox transformation in order to reduce heteroscedasticity.

RESULTS

Pre-dispersal insect infestation of Mucuna holtonii seeds

In total, we collected 1493 seeds from mature *M. holtonii* fruits. The majority of seeds collected prior to fruit pod dehiscence exhibited no signs of insect infestation or fungal damage (Mean per cent \pm 1 SD, undamaged: 95.7% \pm 4.7%, infested by Nitidulidae: 1.5% \pm 3.0%, infested by Rhizophagidae: 1.1% \pm 1.4%, fungus damaged: 1.7% \pm 2.9%). Less than 5% of the seeds were infested by nitidulid and rhizophagid beetles, or infected by fungi ($F = 420$, $df = 3$ $P < 0.0001$).

Vertebrate seed removal and seed fates of Mucuna holtonii

Seeds were removed by vertebrates in 14 out of 15 depots. Most of these seeds were removed within 6 d (Figure 3.1). We were able to record the vertebrate removing each seed, and the fates of 97% of the removed seeds ($N = 90$).

After 16 d in the field, only 18.4% of the seeds escaped vertebrate predation (Figure 3.1). Seeds were moved 0–14.5 m from their initial locations (mean removal distance \pm 1 SD = 1.5 \pm 2.5 m). The majority (98.6%) of these removed seeds were taken by collared peccaries (*Pecari tajacu*, Artiodactyla). Most of the seeds removed by peccaries were eaten (98.6%, Figure 3.1). Only one of the *M. holtonii* seeds removed by a collared peccary escaped predation and was dispersed a distance of 6.95 m from the source. One seed was removed and consumed by a Central American agouti (*Dasyprocta punctata*, Rodentia) during our study. No seeds were removed or handled by other rodent species.

Seed coat as a structural defense against invertebrates in Mucuna holtonii

After 24 h, all seeds with a portion of the seed coat removed were visited by individuals of the neotropical leaf-cutting ant *Sericomyrmex amabilis* Wheeler, 1925 (subfamily Myrmicinae, tribe Attini). These ants collected small pieces of endosperm and transported them to nests for assumed use in fungal cultivation. In subsequent days, we observed *S. amabilis* recruitment and temporary trails used by worker ants to transport *M. holtonii* endosperm into ant nests. *Sericomyrmex amabilis* ants were not able to perforate the seed coats of intact *M. holtonii* seeds. After 16 d, no endosperm was removed from seeds with intact seed coats. All opened seeds were visited by *S. amabilis*, but had varying amounts of endosperm excavated. In seeds with opened seed coats, *S. amabilis* ants removed between 0.5%-100% of endosperm (Mean \pm 1 SD per cent of endosperm removal per depot: Intact seeds = 0%, Opened seeds = 14.5% \pm 17.4%, $t = 2.63$, $df = 9$, $P = 0.027$).

Effects of endosperm removal by invertebrates on Mucuna holtonii seed germination and growth

Endosperm removal reduced the germination success of *M. holtonii* seeds (Logistic regression, range of endosperm removed = 0%–100%, Mean \pm SD = 7.8% \pm 21.4%, $N = 182$ seeds, $\chi^2 = 32.6$, $df = 1$, $P < 0.0001$). Seeds with more than 59.2% of their endosperm removed did not germinate. Endosperm removal also affected shoot and root biomass production in *M. holtonii*. Seeds with large amounts of endosperm removed by *S. amabilis* ants produced less shoot, root, and total (shoot + root) biomass than seeds with small amounts or no endosperm removed (Table 3.1, Figure 3.2).

DISCUSSION

Pre-dispersal seed predation by insects and fungal infection are leading causes of seed death among many plant species (Crawley 1992, Janzen 1971c, Tewksbury et al. 2008). However, pre-dispersal seed attack does not appear to be a major factor in *M. holtonii* seed mortality. For some plant species, physical protection provided by a fruit structure (e.g. thick exocarp) can effectively prevent pre-dispersal seed death from insect predation (Janzen 1971c). During *M. holtonii* seed development, the fruit pod enclosing the seeds likely provides some physical protection against attack from seed predators (e.g. nitidulid and rhizophagid beetles or fungal spores). This protection, however, is ephemeral because *M. holtonii* seeds drop to the forest floor during the ripening process. After *M. holtonii* fruits dehisce and drop mature seeds to the ground, these seeds interact with terrestrial seed predators and face high levels of post-dispersal attack. In our study, we found that the majority of *M. holtonii* seed death occurs on the forest floor rather than on the liana.

At La Selva, collared peccary and Central American agouti are the most abundant terrestrial mammal species (TEAM Network, <http://www.teamnetwork.org/en/>). As generalist frugivores, peccaries and agoutis can either affect seeds of many plant species positively through seed dispersal, or affect seeds negatively via predation (Beck 2005, Bodmer 1991, Jansen *et al.* 2004). Within La Selva, terrestrial mammals have mostly negative effects upon *M. holtonii* seed survival since most of these seeds that fall on the forest floor are consumed and killed by peccaries. As a large seed with protein-rich endosperm (Harms *et al.* 1961), *M. holtonii* is a valuable food resource for peccaries and is thus eaten immediately when encountered by these animals.

Peccaries process food via foregut fermentation. This characteristic allows peccaries to effectively digest toxic seeds such as *M. holtonii* (Carl and Brown 1983, Olmo 1993, Elston *et al.* 2005, Nogueira 2005). Other terrestrial mammals at La Selva, such as rodents, lack complex stomachs capable of pregastric fermentation and cannot overcome the toxicity of these L-dopa-rich seeds (Bell and Janzen 1971). *Mucuna holtonii* seed consumption by rodents during our study was rare, consisting of only a single predation event by one agouti. Therefore, *M. holtonii* seeds are not likely to be handled or dispersed by rodents in the La Selva forest.

Mucuna holtonii seeds face high predation pressure within La Selva because peccaries are locally abundant (E. K. Kuprewicz, unpubl. data). In contrast, agouti and peccary populations are reduced or locally extinct within forest fragments that surround La Selva. In hunted and fragmented forests, a reduction of large terrestrial mammals may increase the number of seeds available for small rodents (Dirzo *et al.* 2007). Additionally, small rodents are less affected by hunting and habitat fragmentation than large mammals, resulting in expected population increases of small rodents after habitat fragmentation ((Dirzo *et al.* 2007)). Because small rodents cannot feed on chemically defended *M. holtonii* seeds, it is possible that these seeds will be more likely to survive and germinate in defaunated forest fragments than within a protected forest like La Selva with high local densities of large mammals.

In many plant species, the seed coat serves as a defense against insect seed predation, preventing insects from boring through to the endosperm and killing the seed's embryo (Janzen 1977a). In *M. holtonii*, the seed coat is very thin, yet it acts as an effective barrier to seed predation by *Sericomyrmex* ants. The great green macaw (*Ara*

ambiguus Bechstein, 1811; Psittacidae) has been observed opening and chewing on fruit pods and eating seeds of *M. holtonii* (D. McClearn pers. comm.). While feeding, these macaws drop many partially eaten seeds to the forest floor. Seeds with opened seed coats, such as those partially eaten by *A. ambiguus*, allow *S. amabilis* ants to access and excavate the seeds' endosperm. Another process that opens the coats of *M. holtonii* seeds is germination. Upon radicle emergence, the seed coat splits and *S. amabilis* ants can access the endosperm within the seed.

Previous work has reported that *Sericomyrmex* ants act as seed predators (Feldmann *et al.* 2000). *Sericomyrmex* ants feed upon seeds of *Parkia panurensis* in the Peruvian Amazon (Feldmann *et al.* 2000). Throughout the course of our study, *S. amabilis* was the only invertebrate seed predator observed to visit and excavate endosperm from *M. holtonii* seeds. *Sericomyrmex amabilis* ants, like other fungus-growing ant species, use collected plant material to cultivate fungus for use as a food resource. It is possible that the cultivated fungus grown by *S. amabilis* ants can digest the toxic chemical compounds found throughout *M. holtonii* seeds, thus rendering this L-dopa-rich endosperm non-toxic and converting it into a productive medium for fungus growth.

For many plant species, loss of seed endosperm results in reduced germination success and subsequent seedling biomass loss (Mack 1998, Vallejo-Marin *et al.* 2006). In our study, endosperm removal by *S. amabilis* ants detrimentally affected the growth of *M. holtonii* seedlings. If attacked seeds were able to germinate, higher amounts of endosperm excavated by *S. amabilis* resulted in less root, shoot, and total (root + shoot) biomass produced by resultant seedlings. Breaching or scarification of *M. holtonii* seed

coats allows opened seeds to more effectively imbibe water and germinate faster than seeds with intact seed coats (Janzen 1977b). However, in the case of *M. holtonii*, scarification of the seed coat allows *S. amabilis* to enter and remove endosperm, thereby reducing the germination success and growth of *M. holtonii*.

In conclusion, the chemical defenses of *M. holtonii* do not offer complete immunity against vertebrates as previous studies have reported (Bell and Janzen 1971, Daxenbichler *et al.* 1972, Harms *et al.* 1961, Janzen 1977b, Janzen *et al.* 1986, Rehr *et al.* 1973). In this study, we observed that seeds of *M. holtonii* are not only chemically defended, but that structural defenses provided by the seed coat can also prevent post-dispersal seed predation by invertebrates. Both chemical and mechanical defenses are fundamental to the survival and propagation of *M. holtonii* throughout tropical rain forests.

Table 3.1. Effect of endosperm removal by *Sericomyrmex amabilis* ants on shoot, root, and total biomass produced by germinating *Mucuna holtonii* seeds ($N = 143$ plants). Linear regressions performed on data after Box-Cox transformations. λ = power transformation parameter of Box-Cox transformation.

	Dry weight (g) Mean \pm 1SD	Range (g)	λ	F	df	r^2	P
Shoot	0.59 \pm 0.40	0 - 1.62	0.82	5.54	1	0.031	0.02
Root	0.11 \pm 0.07	0.0001 - 0.32	-1.22	7.92	1	0.046	0.0056
Total	0.71 \pm 0.47	0.0001 - 1.94	0.78	6.05	1	0.034	0.015

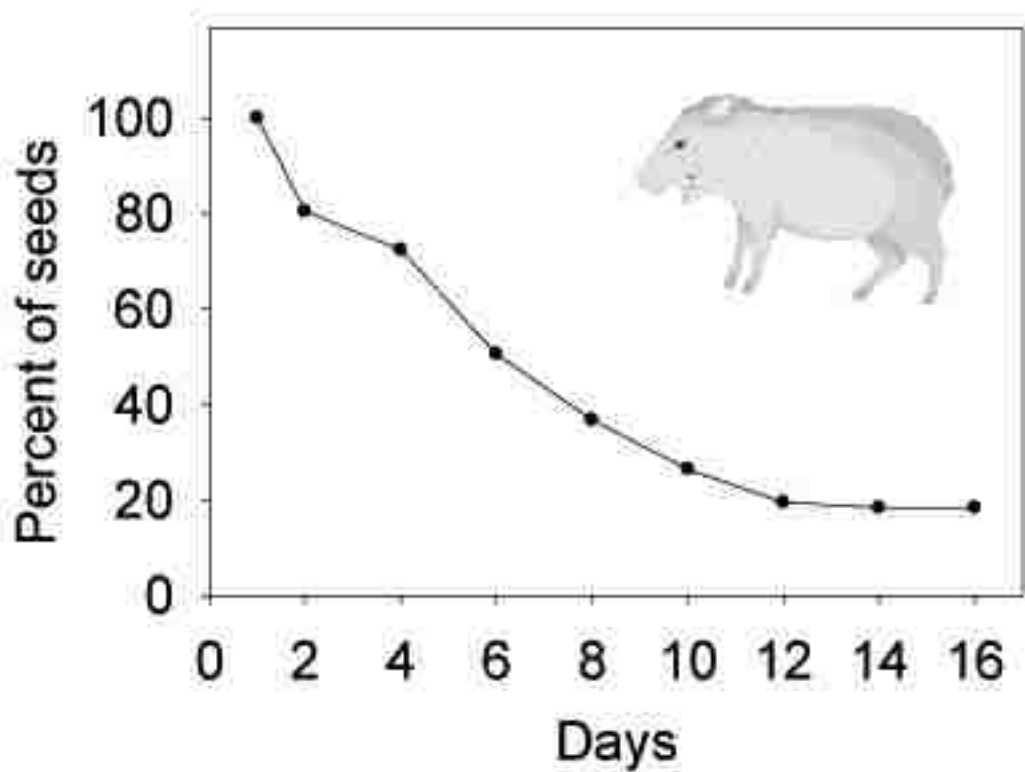


Figure 3.1. Percent survival of *Mucuna holtonii* seeds exposed to mammal seed predators over 16 d. Total number of seeds = 90 (six seeds per independent depot).

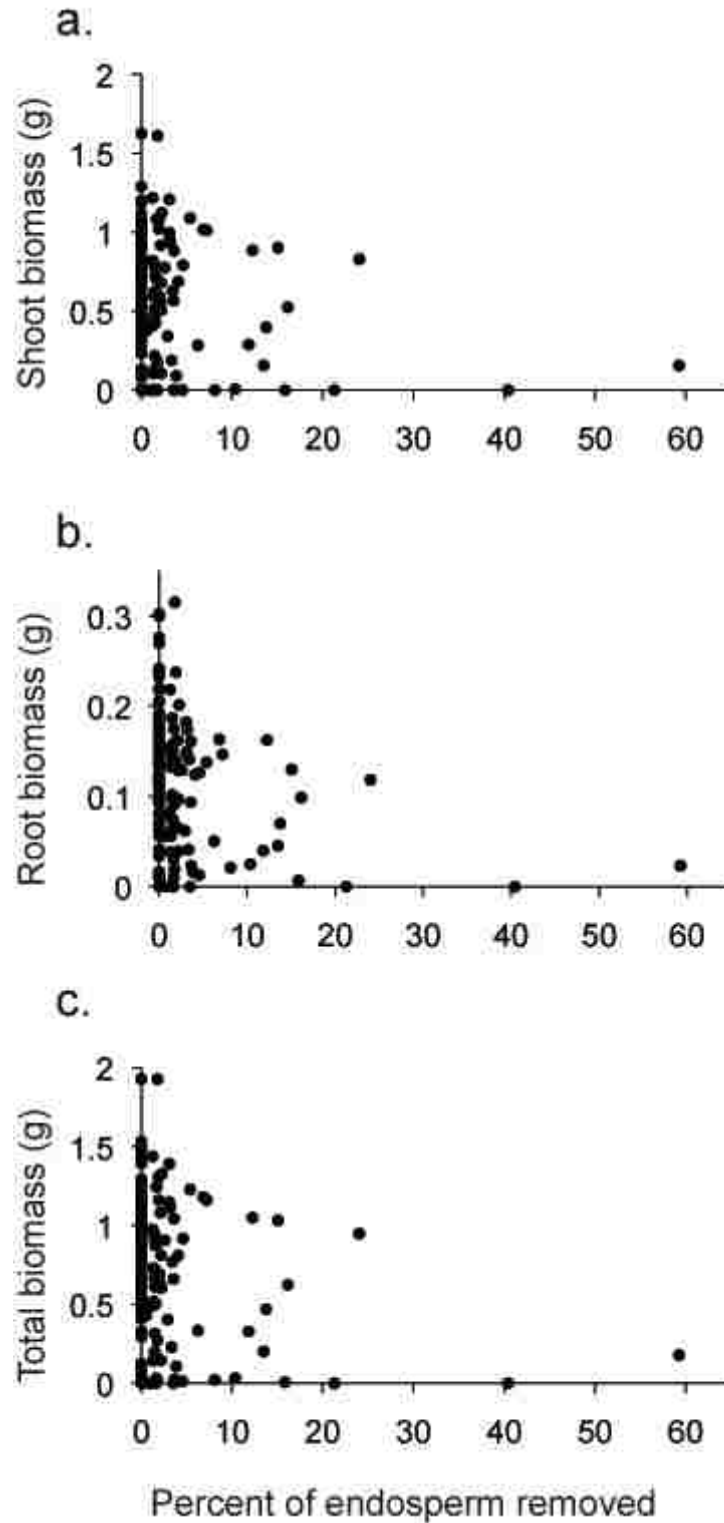


Figure 3.2. Shoot (a), root (b), and total (c) biomass production (dry weights) of *Mucuna holtonii* seedlings from seeds with different percents of endosperm removed by *Sericomyrmex amabilis* ants, $N = 143$ plants.

CHAPTER 4

Affects of scatter-hoarding on large seed survival, germination, and seedling growth

SUMMARY

Central American agoutis (*Dasyprocta punctata*, Rodentia) bury a portion of the seeds they remove singly in shallow subsurface caches – a process known as scatter-hoarding. Although the potential positive effects of scatter-hoarding on seeds have been well-studied, the negative effects of scatter-hoarding on seeds and seedlings are unknown. If hoarded seeds escape predation and become seedlings, they are still susceptible to herbivory by terrestrial mammals. In this study, I tested the hypothesis that scatter-hoarding by agoutis enhances seed survival, germination, and growth by protecting seeds from seed predators. I also determined how terrestrial mammals affected recently germinated seedlings. Using seeds of four large-seeded plants (*Astrocaryum alatum*, *Iriarteia deltoidea*, *Socratea exorrhiza*, and *Dipteryx panamensis*), I created simulated agouti hoards and exposed seeds to invertebrate or vertebrate seed predators for 36 d. I recorded the germination success and growth of seedlings produced from seeds infested and non-infested by *Coccotrypes* beetles. Using mammal enclosures, I recorded the survival of seedlings exposed to or protected from large terrestrial mammals for 140 d. Artificially-hoarded seeds escaped predation by invertebrates and vertebrates while exposed seeds suffered high beetle infestation or almost complete removal by mammals. Hoarding negatively affected seeds of *D. panamensis* by preventing germination. Non-infested palm seeds had higher germination success and produced larger seedlings than seeds infested by *Coccotrypes* beetles. Seedlings of *A. alatum* and *I. deltoidea* suffered high mortality by peccaries and agoutis. Hoarding by

agoutis protected most diaspores from seed predators and enhanced germination success (except for seeds of *D. panamensis*) and seedling growth, although mammals killed many seedlings of two seedling species. Scatter-hoarding by agoutis is beneficial to most seeds and may positively affect plant propagation and resultant seedling survival in lowland Neotropical forests.

BACKGROUND

Seed predation by animals negatively affects plant populations by limiting plant propagation and also may influence forest community structure and plant distributions (Adler and Muller-Landau 2005, Cintra 1997, Clark *et al.* 1999, Crawley 1992, Hulme 1998, Hyatt *et al.* 2003, Silman *et al.* 2003). Once a seed lands on the forest floor, it is susceptible to attack by a suite of terrestrial seed-eating animals including both invertebrates and vertebrates (Janzen 1971c). In Neotropical rain forests, many beetles in the families Bruchidae and Scolytidae attack and kill large seeds, contributing to high levels of seed mortality (Gálvez and Jansen 2007, Janzen 1971c, Notman and Villegas 2005, Silvius and Fragoso 2002). Neotropical rodents (*e.g.* agoutis, squirrels, rats) and ungulates (*e.g.* peccaries, tapirs) also consume, and may potentially disperse, a variety of large seeds (Asquith *et al.* 1997, Beck 2005, Bodmer 1991, Fragoso 1997, Smythe 1986).

Although seed predators negatively affect plant recruitment via seed consumption and destruction, some mammals may positively affect seed survival and propagation by dispersing seeds away from source plants (*e.g.* Forget 1990, Forget 1996, Jansen *et al.* 2004, Smythe 1989). Dispersal away from the parent plant may increase the likelihood of a seed escaping predation (Connell 1971, Howe 1993, Howe and Smallwood 1982,

Janzen 1970, Peres *et al.* 1997). Dispersal may enhance germination success if a seed is deposited in a favorable habitat and subsequently abandoned by the dispersal agent (Howe and Smallwood 1982, Schupp 2007, Wenny and Levey 1998, Wenny 2001).

Some animals disperse seeds by hoarding them in subsurface caches, conserving them for future use (Jansen *et al.* 2004, Vander Wall 1990). Consumption of a hoarded seed is temporally deferred and the cached item is transported, deposited, and concealed in a way that prevents detection by kleptoparasitic seed predators ((Hallwachs 1986)). Scatter-hoarders store single food items within numerous caches located throughout their home ranges (Gálvez *et al.* 2009, Morris 1962). Previous research on scatter-hoarding by mammals has focused only on the positive effects that hoarding has on seeds (*e.g.* Donatti *et al.* 2009, Hallwachs 1986, Smythe 1989, Vander Wall 1990, Wenny 2001) while potential damaging effects of hoarding have not been documented. Hoarded seeds are usually dispersed long distances from parent trees, enhancing the probability that these seeds will escape detection by other foraging granivores (Hallwachs 1986, Smythe 1989, Vander Wall 1990). Scattered, shallow caches also hide plant propagules from seed predators and may provide potentially favorable microsites for seed germination and seedling establishment (Asquith *et al.* 1999, Briggs *et al.* 2009, Forget 1990, Vander Wall 1990). Scatter-hoarding, however, also may negatively affect seeds if they are hoarded in unsuitable habitats or buried too deep within the soil, preventing germination and seedling emergence (Vander Wall 1990).

Central American agoutis (*Dasyprocta punctata* Gray, 1842; Rodentia) are large (2 – 4 kg) terrestrial mammals that consume, disperse, and scatter-ward seeds of numerous large-seeded plants (Hallwachs 1986, Smythe 1978). Agouti scatter-hoarding

behavior has been well-documented (Gálvez *et al.* 2009, Hallwachs 1986, Smythe 1978), but few studies have quantified the positive and negative effects that this hoarding activity may have on seed survival, germination, and resultant seedling growth.

Plant mortality is usually highest during the seed and young seedling stages (Clark and Clark 1985, Silman *et al.* 2003). Seedlings are susceptible to damage and destruction by large terrestrial mammals in the understory via trampling (Clark and Clark 1989), herbivory (Asquith *et al.* 1997, Clark and Clark 1989, DeMattia *et al.* 2006), or seed predation (Brewer and Webb 2001, Pyare and Longland 2000, Smythe 1989). Understory ungulate species that forage in large groups (*e.g.* collared peccaries, *Pecari tajacu*, Linnaeus, 1758; Artiodactyla) can trample or incidentally bury seedlings while searching for food on the forest floor. Mammal herbivores can also directly forage for and consume seedlings (Asquith *et al.* 1997, DeMattia *et al.* 2006). Small seedlings may act as indicators of below ground seed presence, providing mammals with a cue to this food source, which they then excavate and remove the attached seed resulting in seedling death (Brewer and Webb 2001, Pyare and Longland 2000, Smythe 1989).

In this study I test the hypothesis that scatter-hoarding by *D. punctata* positively affects seeds by protecting them from seed predators and by potentially enhancing interred seed germination and resultant seedling growth. However, once seeds germinate and young seedlings emerge on the forest floor, they are no longer hidden from seed predators, and seedlings and seeds still attached to seedlings are susceptible to attack by large generalist mammal herbivore-granivores. To test these hypotheses, the main objectives of my research were: (1) to determine if hoarding by agoutis effectively protects plant propagules from detection and destruction by invertebrate and vertebrate

seed predators, (2) to assess the germination success and growth of seedlings from palm seeds (*Astrocaryum alatum*, *Iriartea deltoidea*, *Socratea exorrhiza*) infested by invertebrates (exposed on the soil surface) versus non-infested seeds (hoarded), (3) to ascertain if hoarding by agoutis positively or negatively affects the germination of *Dipteryx panamensis* seeds, and (4) to determine how large terrestrial mammals affect the survival of young, recently-germinated seedlings.

METHODS

Study site

This study was conducted from November 2006 to December 2008 at La Selva Biological Station (henceforth La Selva) located in Puerto Viejo, Sarapiquí, Heredia, Costa Rica (10°26' N, 83°59' W). La Selva is a protected reserve classified as tropical lowland wet forest (Holdridge *et al.* 1971), which comprises 1600 ha of old-growth forest, secondary growth, swamps, and tree plantations. La Selva receives approximately 4000 mm of rainfall per year and is aseasonal, having no distinct dry season (McDade *et al.* 1994). I conducted the following study throughout the primary forest of La Selva, corresponding to the habitat where *Astrocaryum alatum*, *Iriartea deltoidea*, *Socratea exorrhiza*, and *Dipteryx panamensis* are most abundant.

Study species

For hoarding experiments, I used fresh, ripe seeds of *A. alatum*, *I. deltoidea*, *S. exorrhiza* (all Arecaceae), and entire fruits of *D. panamensis* (Fabaceae) (Table 4.1). Agoutis hoard *D. panamensis* seeds while they are still enclosed within fruit endocarps

(Forget 1993, pers. obs.). All of these large seeds and fruits are abundant throughout the primary forest of La Selva and are readily eaten by agoutis, peccaries, squirrels, and small rodents and are hoarded by agoutis (pers. obs.). Agoutis and peccaries do not pass any of these large diaspores (here used to refer to plant dispersal units, seeds for *A. alatum*, *I. deltoidea*, *S. exorrhiza* and fruits of *D. panamensis*) through their guts intact (endozoochory), rather they grind seeds prior to ingesting them. Predation of seeds of *A. alatum*, *I. deltoidea*, *S. exorrhiza*, and *D. panamensis* by agoutis and peccaries results in the complete destruction of seed embryos.

The effects of hoarding on seed detection by invertebrate and vertebrate seed predators

In the hoarding experiments, I collected ripe fallen seeds and fruits during the peak fruiting seasons for each species. I collected fruits from at least 15 individual trees per species. Seeds were thereafter pooled (by species) and a subset was randomly chosen for use in hoarding experiments. Hoarding experiments were completed during the months when each respective tree species was fruiting so as to coincide with the time period when ambient abundances of fruits for each seed species were highest.

For all experiments, I used seeds of similar sizes and masses (of each species) with no evidence of insect infestation or fungus growth. To detect insect infestation, I visually inspected each seed for small holes (indicative of infestation by scolytid beetles) or large holes (indicating infestation by bruchid beetles); any seeds with apparent holes were discarded.

Prior to placement in the field, I removed all pulp from each seed of *A. alatum*, *I. deltoidea*, and *S. exorrhiza* to mimic agouti hoarding behavior for these species (pers.

obs.). However, for *D. panamensis*, agoutis hoard the entire fruit pod (exocarp, endocarp, and enclosed seed; pers. obs.), therefore I left these fruits intact for placement in the field. Twelve seeds of a single species were placed in each hoarding depot; six seeds were hoarded (buried) 5 cm beneath the soil (mean depth of agouti seed hoards, Smythe 1978) and six seeds remained exposed on the soil surface arranged in a line perpendicular to the line of buried seeds (Fig. 4.1). Each seed was separated from others by 2 cm. I lightly pressed the soil down over hoarded seeds to mimic the soil compacting behavior of hoarding agoutis (Smythe 1978, pers. obs.). I covered seeds and fruits at a portion (45%) of the hoarding depots with a wire mesh cage (length = 30 cm, width = 30 cm, height = 5 cm, mesh size = 2 × 2 cm) staked firmly into the soil to prevent seed removal by mammals but allowing insects access to the seeds (invertebrate treatment) (Fig. 4.1). Identical seed arrangements without cages were open to seed-eating mammals (vertebrate treatment). Hoarding depots (either invertebrate or vertebrate treatment) were placed singly throughout the primary forest and separated by a minimum distance of 300 m to prevent agouti home range overlap with more than one depot (agouti home range estimates are smaller than 300 m; Smythe 1978, Dubost 1988). I placed the following numbers of hoarding depots (caged, invertebrate access treatment / non-caged, vertebrate access treatment) throughout the primary forest of La Selva for each diaspore species: *A. alatum*: 10 / 11; *I. deltoidea*: 10 / 10; *S. exorrhiza*: 10 / 18; and *D. panamensis*: 10 / 10.

All hoarding depots (both invertebrate and vertebrate treatments) remained in the field for 36 d (seeds on forest floor are encountered by terrestrial mammals and insects within this time, Chapter 1, this dissertation). Thereafter, I assessed seed and fruit infestation by insects in the caged, invertebrate access treatment and removal by

mammals in the non-caged, vertebrate access treatment (seed presence or absence). I compared infestation by insects and removal by mammals for all hoarded and non-hoarded seeds and fruits using paired t-tests. I collected all intact seeds and fruits from the field for germination and growth experiments.

Germination success and seedling growth of palm seeds infested by invertebrates

I planted infested and non-infested seeds of *A. alatum*, *I. deltoidea*, and *S. exorrhiza* in individual germination bags filled with soil gathered from a single location within the primary forest to ensure a consistent growth medium for all seeds. *Dipteryx panamensis* fruits were not infested by invertebrates so this species was excluded from this portion of the study. Seeds were exposed to homogenous natural light, rain, and soil conditions within the shade house (76% shade). I recorded germination success (production of radicle and plumule) of infested and non-infested seeds for all palm species. I compared germination success of infested and non-infested palm seeds using Fisher exact tests (for 2×2 matrices of count data for each species). I also measured seedling heights after a growth period of 120 d and I compared the heights of seedlings (= length of longest leaf) from infested and non-infested *S. exorrhiza* seeds (the only palm species in which infested seeds germinated) using a Welch two sample t-test (a form of the t-test used for samples that have unequal variances).

Germination success of hoarded and non-hoarded D. panamensis seeds

Dipteryx panamensis seeds germinate rapidly relative to the aforementioned palm seeds, therefore I assessed how hoarding by agoutis affects germination success, rather

than seedling growth, of this species. After exposure to animals in the field, I brought all hoarded and exposed *D. panamensis* fruits to a shade house to prevent further animal interference. Depending on their previous state in the field (hoarded or non-hoarded), fruits were either buried 5 cm below the soil (hoarded) or placed on the soil surface (non-hoarded) for an additional 14 days to allow adequate time for seedling germination (no seeds had germinated within 36 d). I recorded germination success for each diaspore after 50 days. I compared the germination success of hoarded versus non-hoarded *D. panamensis* seeds after 50 days using Fisher exact tests (for 2×2 matrices of count data).

The effects of large terrestrial mammals on young seedling survival

For seedling experiments, I grew seedlings from freshly harvested seeds of *A. alatum*, *I. deltoidea*, *S. exorrhiza*, and *D. panamensis*. To determine how large terrestrial mammals (e.g. *D. punctata* and *P. tajacu*) affect the survival of these large-seeded tree seedlings, I planted seedling pairs of *A. alatum*, *I. deltoidea*, *S. exorrhiza*, and *D. panamensis* throughout the primary forest of La Selva. All seedlings of each species were of similar ages and sizes when they were planted in the field. *Astrocaryum alatum*, *I. deltoidea*, and *S. exorrhiza* seedlings still retained nutrient sources (seeds) when they were placed in the field, and *D. panamensis* seedlings retained their cotyledons when they were planted in the forest.

To determine how large terrestrial mammals affect seedling survival, I placed *A. alatum*, *I. deltoidea*, *S. exorrhiza*, and *D. panamensis* seedlings within and outside mammal exclosures and monitored their survival. One seedling of each pair was enclosed within a cylindrical mammal exclosure made of metal mesh (diameter = 25 cm;

height = 1 m; mesh size = 2 x 2 cm); the other seedling was planted 1 m from the caged seedling and remained open and exposed (non-caged) to all seedling predators. Mammal enclosures prevented access by all terrestrial mammals, but allowed access to seedlings by invertebrates, fungus spores, and pathogens. Paired seedlings were separated from other pairs by at least 300 m to ensure independence.

I checked seedling pairs every 14 d and noted survival status (alive or dead) of each caged and non-caged seedling. All seedling pairs had similar rates of growth and leaf production. I monitored seedling pairs for a total of 140 d. I compared the numbers of surviving caged and non-caged seedlings at the end of the 140 d observation period using chi-squared tests.

RESULTS

The effects of hoarding on seed detection by invertebrate and vertebrate seed predators

After 36 d exposure on the primary forest floor, seeds from all palm species (*A. alatum*, *I. deltoidea*, and *S. exorrhiza*) were infested by beetles of the genus *Coccotrypes* (Coleoptera: Scolytidae). These small (approximately 1 mm in length) beetles bored holes through the seed coat and into the endosperm of the palm seeds. Hoarded *A. alatum* seeds were protected from infestation by invertebrate seed predators whereas non-hoarded seeds suffered significantly higher levels of infestation by *Coccotrypes* beetles ($t = -2.25$, $df = 10$, $P < 0.048$, $N = 11$ depots) (Figure 4.2a). Hoarded *I. deltoidea* seeds had significantly lower levels of invertebrate infestation than non-hoarded seeds ($t = -4.45$, $df = 9$, $P < 0.002$, $N = 10$ depots) (Figure 4.2b). Hoarded *S. exorrhiza* seeds also had significantly lower levels of infestation by *Coccotrypes* beetles than non-hoarded seeds (t

= -16.10, $df = 9$, $P < 0.001$, $N = 10$ depots) (Figure 4.2c). There was no evidence of infestation by *Coccotrypes* beetles or other insects in any of the hoarded or non-hoarded *D. panamensis* fruits (Figure 4.2d).

The large sizes of all propagules used in this study precluded their removal by small mammals (e.g. *Heteromys*, *Proechimys*); all mammal interaction with seeds was by peccaries and agoutis. After 36 d, these large mammal frugivore-granivores had visited all hoarding depots (vertebrate treatment) and removed diaspores of all four plant species (*A. alatum*, *I. deltoidea*, *S. exorrhiza*, and *D. panamensis*). For all seeds and fruits, hoarded seeds were effectively hidden from detection and subsequent removal by mammals. Hoarded *A. alatum* seeds had significantly lower levels of removal by vertebrates than non-hoarded seeds ($t = -2.69$, $df = 9$, $P < 0.025$, $N = 10$ depots) (Figure 4.3a). Hoarded *I. deltoidea* seeds had significantly lower levels of removal by mammals than non-hoarded seeds ($t = -7.632$, $df = 9$, $P < 0.001$, $N = 10$ depots) (Figure 4.3b). Hoarded *S. exorrhiza* seeds had significantly lower levels of removal by vertebrates than non-hoarded seeds ($t = -107.00$, $df = 17$, $P < 0.001$, $N = 18$ depots) (Figure 4.3c). Hoarded *D. panamensis* fruits had significantly lower levels of removal by mammals than non-hoarded fruits ($t = -12.53$, $df = 9$, $P < 0.001$, $N = 10$ depots) (Figure 4.3d).

Germination success and seedling growth of palm seeds infested by invertebrates

Palm seeds (of *A. alatum*, *I. deltoidea*, and *S. exorrhiza*) that were infested by *Coccotrypes* beetles had low germination success. Infested seeds of *I. deltoidea* and *S. exorrhiza* had lower germination success than seeds that had not been attacked by *Coccotrypes* (*I. deltoidea*: Fisher exact test, $P < 0.001$; *S. exorrhiza*: Fisher exact test, $P <$

0.001) (Figure 4.4b, c). In contrast, infested *Astrocaryum alatum* seeds did not have significantly lower levels of germination success than non-infested seeds (Fisher exact test, $P = 0.11$) (Figure 4.4a).

Of the three palm seed species used in this study, only infested *S. exorrhiza* seeds germinated and produced seedlings within 120 d of observation. Seedlings produced from *S. exorrhiza* seeds infested by *Coccotrypes* beetles were shorter than seedlings produced from non-infested seeds (Welch two sample t-test, $t = -8.29$, $df = 120$, $P < 0.001$) (Figure 4.5).

Germination success of hoarded and non-hoarded D. panamensis seeds

Hoarding had a detrimental effect on the germination success of *D. panamensis* propagules resulting in fewer seeds germinating from hoarded fruits than from non-hoarded fruits after 50 d (Fisher exact test, $P < 0.001$) (Figure 4.6).

The effects of large terrestrial mammals on young seedling survival

Some seedling species exposed to peccaries and agoutis had high survival while some species were killed by granivores. One hundred percent of seedlings protected from mammals (caged *A. alatum*, *I. deltoidea*, *S. exorrhiza*, and *D. panamensis*) survived through the 140 d study period. Seedlings of *A. alatum* and *I. deltoidea* that were exposed to agoutis and peccaries suffered higher levels of predation than caged seedlings (*A. alatum*: Chi-squared test, $\chi^2 = 7.35$, $df = 1$, $P < 0.007$; *I. deltoidea*: Chi-squared test, $\chi^2 = 17$, $df = 1$, $P < 0.001$) (Figure 4.7). In contrast, very few non-caged seedlings of *S. exorrhiza* and *D. panamensis* were killed by mammal herbivores; the number of

seedlings surviving at the end of the study did not significantly differ between caged and non-caged seedling treatments (*S. exorrhiza*: Chi-squared test, $\chi^2 = 0.47$, $df = 1$, $P = 0.49$; *D. panamensis*: Chi-squared test, $\chi^2 = 0.11$, $df = 1$, $P = 0.75$) (Figure 4.7).

DISCUSSION

Most research concerning how hoarding by animals affects plant propagules has only focused on how hoarding positively affects seeds (Hallwachs 1986, Smythe 1989, Vander Wall 1990, Wenny 2001) while the negative aspects of hoarding have been unstudied (but see Jansen *et al.* 2006). I found that hoarding by agoutis was beneficial to most of the tree species used in this study, however, hoarding also negatively affected some aspects of propagule survival.

In this study, simulated hoarding by agoutis effectively protected seeds of all three palm species (*A. alatum*, *I. deltoidea*, and *S. exorrhiza*) from detection and subsequent infestation by invertebrates. All observed invertebrate infestation was by scolytid beetles from the genus *Coccotrypes*. Females of *Coccotrypes* spp. lay 1 – 100 eggs within a single palm seed (Notman and Villegas 2005). Seed death from this infestation likely depends on location and intensity of attack by *Coccotrypes*. Beetles that bore through the seed embryo will likely kill it, preventing the seed from germinating, whereas beetles that bore through endosperm only remove nutrient source and seed viability remains intact (Mack 1998, Mendoza and Dirzo 2009, Vallejo-Marín *et al.* 2006). Also, infested seeds that contain high numbers of beetles are less likely to germinate and produce seedlings than seeds infested with low numbers of *Coccotrypes* individuals (Notman and Villegas 2005). Palm seed infestation by *Coccotrypes* beetles

occurs on the forest floor rather than in the canopy (post-dispersal seed predation). This is likely because of inability of these small beetles (1 mm length) to penetrate exocarp and pulp present on unripe fruits of *A. alatum*, *I. deltoidea*, and *S. exorrhiza* in the canopy (Janzen 1971c). Once these fruits mature, they shed their exocarp and pulp and seeds fall to the ground where they are exposed to *Coccotrypes* infestation (Kirkendall *et al.* 1997). Contrary to expectation, no infestation by bruchid beetles, a common invertebrate predator of large seeds in lowland Neotropical forests (Janzen 1971c, Silvius 1999, Silvius and Fragoso 2002, Wright 1983), was observed in seeds during this study.

Seeds of *A. alatum*, *I. deltoidea*, and *S. exorrhiza* benefited from hoarding as very few seeds of these species (no seeds in the case of *S. exorrhiza*) were discovered and attacked by *Coccotrypes* beetles. Hoarding had no apparent effect on levels of *D. panamensis* fruit infestation by invertebrate seed predators because no fruits of this species, either hoarded or non-hoarded, were infested. It is likely that *Coccotrypes* beetles were unable to penetrate the thick (5 – 7 mm), stony endocarp of *D. panamensis* fruit pods and gain access to the oily pulp and seed within. With the exception of *D. panamensis*, hoarding by agoutis has the potential to protect seeds from invertebrate attack, thereby allowing hoarded palm seeds to escape detection by insects, potentially germinate, and grow if they remain not recovered by the hoarder.

Hoarding positively affected all seed species used in this study by effectively protecting buried diaspores from detection and removal by vertebrate seed predators. At La Selva, Central American agoutis and collared peccaries are the most abundant terrestrial mammal frugivores within the primary forest (TEAM Network, <http://www.teamnetwork.org/en/>). Agoutis consume and potentially disperse large seeds

via hoarding, whereas peccaries consume and destroy most of the large seeds that they encounter on the forest floor (Beck 2005). Although peccaries and other Neotropical ungulates can disperse seeds that pass through their guts intact (Beck 2005), in this study, peccaries basically had a negative impact on propagules of *A. alatum*, *I. deltoidea*, *S. exorrhiza*, and *D. panamensis* through predation because seeds of these tree species are too large for endozoochorous dispersal (see Chapter 2 of this dissertation).

Mammals primarily locate food via olfaction (Price and Jenkins 1986). Because very few hoarded seeds were uncovered and removed by foraging mammals, it is likely that the depth within the soil that agoutis hoard seeds sufficiently obscures the scents of these food items. Previous research has found that leaf litter also may serve to obscure large seed and fruit odor cues, preventing seed predation by agoutis, peccaries, and other frugivorous mammals (Cintra 1997). Agoutis and peccaries also use visual cues, such as the seeds themselves and disturbed soil, to detect food items on the forest floor (pers. obs.) and diaspore burial obscures and protects seeds from mammals that scan the understory during foraging bouts.

Although parent trees suffer high seed losses by large groups of foraging peccaries (up to 30 individuals, pers. obs.), the soil disturbance that occurs during these bouts may result in the incidental burial of some seeds (Clark and Clark 1989). If seeds are sufficiently covered by soil, they may benefit from burial and escape subsequent detection by invertebrate and vertebrate seed predators.

Seeds not protected from invertebrates (by hoarding) had high levels of infestation and low germination success. No infested seeds of *A. alatum* or *I. deltoidea* successfully germinated and only 8% of *S. exorrhiza* seeds infested by *Coccotrypes*

beetles germinated after 120 d. Hoarding indirectly improved palm seed germination success because hoarded seeds were likely not infested. Although germination of infested seeds of *A. alatum* did not differ significantly from non-infested seed germination, this result is likely an artifact of small sample size. Very few (N = 4) *A. alatum* seeds placed in the field were infested by scolytid beetles, resulting in only four infested seeds for use in germination observations. The thick endocarp (5 mm) of *A. alatum* palm seeds prevented most attempts by *Coccotrypes* to bore through to the endosperm. I observed that although many non-hoarded *A. alatum* endocarps contained bore holes typical of *Coccotrypes* attack, only rarely did these holes penetrate completely to the seed endosperm. The physical protection provided by the thick endocarp surrounding *A. alatum* seeds may exclude most *Coccotrypes* beetles that are not large enough to bore through this structural defense.

Loss of seed endosperm may not result in seed death (nonlethal predation), but seeds with large amounts of endosperm removed by invertebrates have lower germination success and smaller resultant seedlings than intact, non-infested seeds (McHargue and Hartshorn 1983, Vallejo-Marín *et al.* 2006, Kuprewicz and García-Robledo 2010). Although this incomplete predation may not prevent germination, insect attack does open the seed coat and allows access to endosperm by fungal pathogens that can kill seeds. Partial seed predation may also prevent seed germination if opened seeds dehydrate (Vallejo-Marín *et al.* 2006). *Coccotrypes* beetles prevented germination of *A. alatum*, *I. deltoidea*, and *S. exorrhiza* seeds by directly boring through the seed embryo, by removing large amounts of endosperm, or by opening the seed to fungal attack and subsequent death. Partial endosperm removal from seeds of *S. exorrhiza* by *Coccotrypes*

resulted in stunted growth of seedlings because these insects removed the nutrient source available for seedling development. For this tree species, it is likely that seeds with high amounts of endosperm removed by *Coccotrypes* beetles produce shorter seedlings than seeds with small amounts of endosperm removed by these beetles.

Hoarding of *D. panamensis* fruits negatively affected seed germination success. Non-hoarded fruits exposed on the soil surface had high germination success (89%) while only 7% of hoarded seeds germinated. All non-germinating seeds had rotted within the fruit pod after 50 d of burial 5 cm below the soil. Previous studies have found that seed germination and seedling emergence are hindered if seedlings cannot penetrate the deep soil or leaf litter under which they are buried (Chambers and MacMahon 1994, Hamrick and Lee 1987, Vázquez-Yanes and Orozco-Segovia 1993). It is likely that at the mean depth of agouti hoards, pressure exerted by the compacted soil atop *D. panamensis* diaspores prevented fruit dehiscence and radicle and plumule emergence, trapping excess moisture within the unopened pod, and rotting the seed.

In this study, the survival of seedlings exposed to mammals differed among plant species. While peccaries and agoutis killed non-caged seedlings of all four tree species, only seedlings of *A. alatum* and *I. deltoidea* had significantly different levels of mortality between caged (protected from mammals) and non-caged (exposed to mammals) treatments. Most non-caged seedlings died from uprooting and subsequent seed predation as opposed to trampling or herbivory by terrestrial mammals. In the La Selva forest, as demonstrated in previous studies, small seedlings may be acting as cues signaling underground food resources (seeds) for mammal granivores (Brewer and Webb 2001, Pyare and Longland 2000, Smythe 1989). For some seedling species, removal of

an attached seed may not result in seedling death (Mack 1998), however uprooting and seed removal by peccaries and agoutis of seedlings of *A. alatum* and *I. deltoidea* resulted in death for 100% of the affected plants.

In tropical rain forests, invertebrates play a major role in the herbivory and subsequent mortality of young seedlings (Agrawal and Fishbein 2006, Boege and Marquis 2005, Clark and Clark 1985, Coley and Barone 1996). In the present study, seedlings within mammal exclosures were accessible to invertebrates and fungal pathogens. At the end of 140 d, 100% of all caged seedlings had survived and retained no evident meristem or leaf damage by invertebrate herbivores. Surprisingly, in my study, insects and fungal pathogens had negligible effects on the survival of young seedlings of *A. alatum*, *I. deltoidea*, *S. exorrhiza*, and *D. panamensis*. Perhaps young seedlings of these species possess tough leaves or secondary metabolites that deter fungus and insect attack (Coley and Barone 1996).

To prevent herbivory by mammals, some seedlings exhibit physical or chemical defenses (Coley and Barone 1996, Freeland and Janzen 1974, Gurevitch *et al.* 2006). *Astrocaryum alatum* seedlings possess spines covering their leaves and stems. Although, spines are generally thought to deter herbivory by mammals (Gurevitch *et al.* 2006), the spines of *A. alatum* seedlings offered little defense against seed predation and subsequent seedling destruction by agoutis and peccaries (72% of seedlings exposed to mammals were killed within 140 d). Terrestrial mammalian granivores were able to bypass these physical defenses, uproot young plants, remove attached seeds, and incidentally kill exposed seedlings.

After an exposure period of 140 d, all seedlings of *I. deltoidea* exposed to mammals were killed. Seedlings of this tree species have no spines or apparent physical defenses against herbivory aside from thick leaves that may decrease palatability (Coley and Barone 1996, Gurevitch *et al.* 2006). It is interesting to note that in this study, all palm seedling deaths occurred due to predation of attached seeds and resultant seedling uprooting rather than by direct herbivory (*i.e.* consumption of meristem or leaf area). Young leaves of these seedlings may possess physical or chemical anti-herbivory defenses. In contrast, seedlings of *D. panamensis* killed by mammals ($N = 2$) died from leaf and cotyledon consumption. Only 10% of *D. panamensis* seedlings available to mammals were consumed and killed within the census period. The low mortality of *D. panamensis* seedlings observed in this study contrasts with very high mortalities found in previous research (81% mortality in 12 mo, Coley and Barone 1996, Clark and Clark 1989; 67% – 88% in 13 mo, De Steven and Putz 1984). The low amount of vertebrate herbivory on young *D. panamensis* seedlings observed in this study may be attributed to the low densities of conspecific seedlings near the experimental pairs. Seedling pairs were separated by at least 300 m and were not located near naturally occurring seedlings of *D. panamensis*. Previous studies have found that seedlings of this species experience high density-dependent mortality (Clark and Clark 1985, Clark and Clark 1984, De Steven and Putz 1984) and it is likely that the seedling densities of *D. panamensis* created by this study were too low to elicit a strong herbivore response.

This study reinforces the idea that the seed and seedling stages of plants are highly susceptible to mortality from seed-eating insects and mammals. Seeds and seedlings not protected from invertebrate and vertebrate seed predators suffered high

mortality rates. Scatter-hoarding by agoutis positively affected palm seeds by protecting them from both insect and mammal predation, thereby improving subsequent seed germination and seedling growth. However, for *D. panamensis* propagules, there is a trade-off in survival: hoarding protects fruits from vertebrate predation but prevents seed germination. Although *D. panamensis* fruits hoarded by agoutis have a higher likelihood of surviving than non-hoarded fruits, buried seeds are unlikely to germinate and produce seedlings that can emerge from the soil. This unexpected outcome demonstrates that hoarding cannot be assumed always to purely benefit plant propagules, as numerous past studies have asserted. It also has negative effects on some plant diaspores that cannot germinate in agouti caches.

Surprisingly, invertebrates and fungi played no role in seedling mortality within 140 d. While seedling species were differentially affected by mammal herbivores, all palm seedling death was attributed to seed predation and seedling uprooting rather than herbivory. Although leaves of *A. alatum*, *I. deltoidea*, and *S. exorrhiza* may be unpalatable to mammal herbivores, seedlings serve as indicators of subsurface seeds that are attractive to these animals. In conclusion, scatter-hoarding can be beneficial or detrimental to diaspores by having a significant effect on rates of seed infestation and predation by mammals, germination success, and seedling growth. Agouti hoarding behavior has the potential to strongly influence seed and seedling survival and potential tree establishment in Neotropical forests where these mammals are common or abundant.

Table 4.1. Tree, seed, and fruit characteristics of all plant species used in hoarding experiments. Sample sizes for masses: *Astrocaryum alatum* $N = 104$, *Iriartea deltoidea* $N = 50$, *Socratea exorrhiza* $N = 69$, *Dipteryx panamensis* $N = 14$.

Species	Family	Local tree density (stems/ha)	Fruiting period	Mass (g) Mean \pm 1 SD	Diaspore dimensions
<i>Astrocaryum alatum</i>	Arecaceae	30.5 ₁	year-round	25.1 \pm 4.5	length = 6 cm, width = 4 cm
<i>Iriartea deltoidea</i>	Arecaceae	22.7 ₂	year-round	2.9 \pm 0.9	diameter = 2-2.8 cm
<i>Socratea exorrhiza</i>	Arecaceae	36.7 ₂	year-round; peak Oct. – Dec.	3.6 \pm 0.6	length = 2.5-3.5 cm, diameter = 1.5-2 cm
<i>Dipteryx panamensis</i>	Fabaceae	0.7 ₂	Nov.- March	32.0 \pm 4.3	length = 6 cm, width = 3 cm

₁(Hartshorn 1983), ₂(Lieberman and Lieberman 1987)

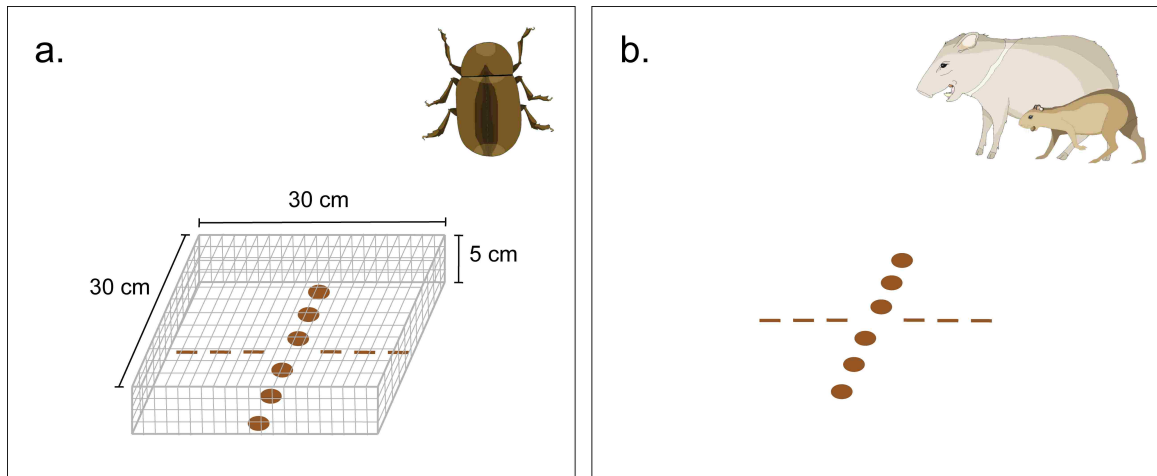


Figure 4.1. Layout of seeds in hoarding depots for the invertebrate treatment (a) where an enclosure prevents mammal access to seeds yet allows invertebrates access, and vertebrate treatment (b) which has no enclosure allowing mammals to access and remove seeds. Six seeds were placed on the soil surface (symbolized here by brown ovals) and six seeds were buried 5 cm below the soil to mimic agouti scatter-hoards (symbolized here by brown dashes). Each depot (either a single invertebrate or vertebrate treatment) was placed within the primary forest at least 300 m from other depots.

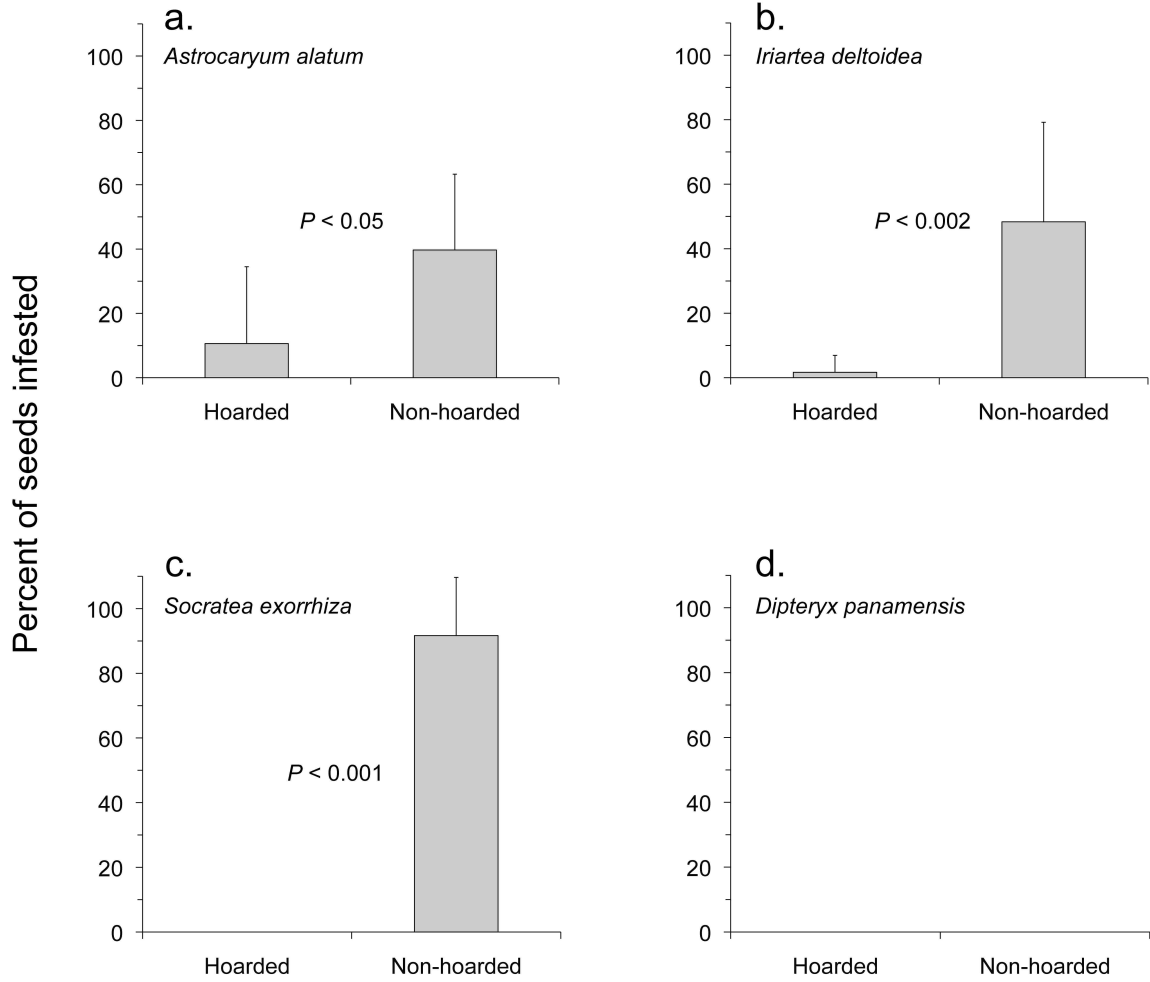


Figure 4.2. Invertebrate infestation (mean percent of seeds infested in each depot + 1 SD) of seeds after either 50 d of burial under 5 cm of soil (hoarded) or exposure on the soil surface (non-hoarded) for seeds of *A. alatum* (a), *I. deltoidea* (b), *S. exorrhiza* (c), and fruits of *D. panamensis* (d). $N = 10$ depots for each seed species.

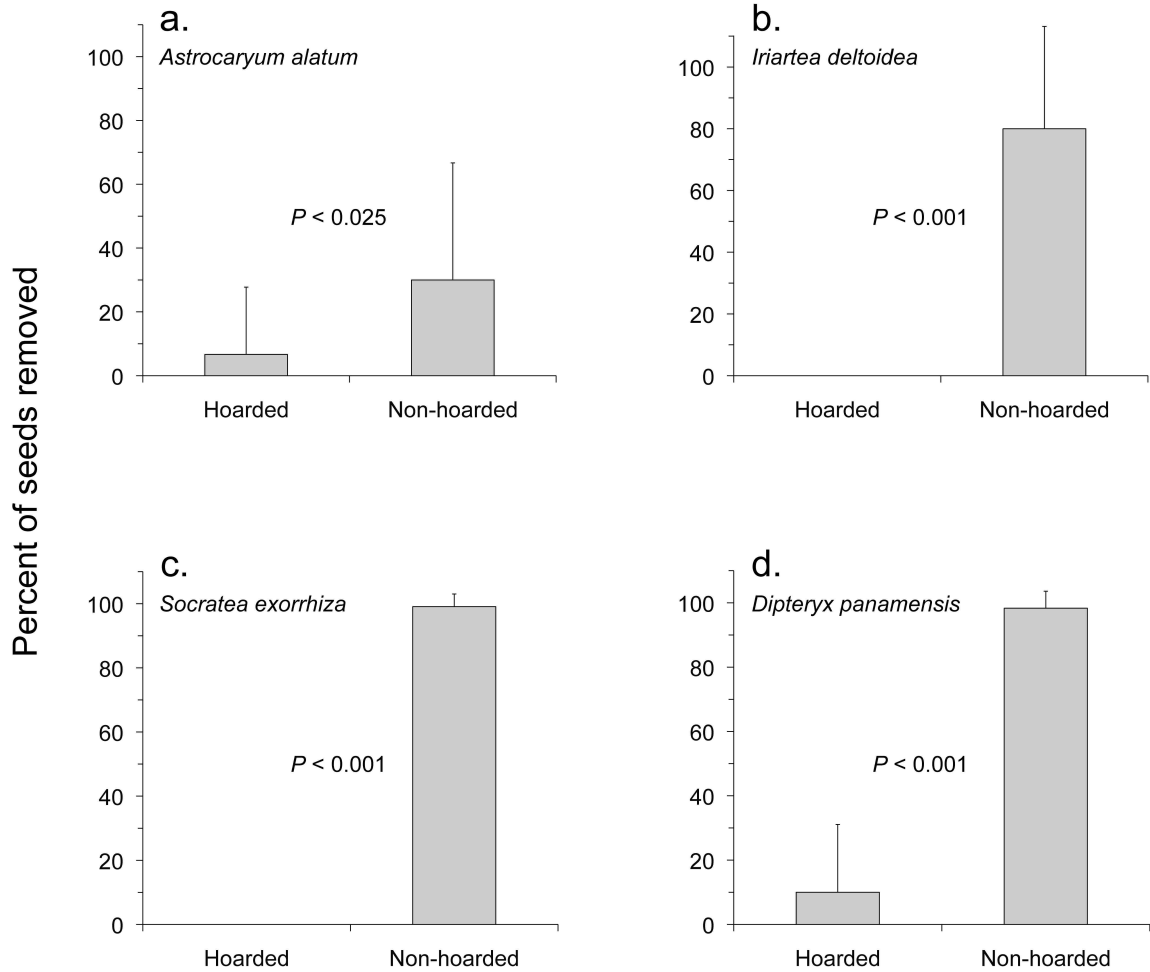


Figure 4.3. Removal by terrestrial mammals (mean percent of seeds removed from each depot + 1 SD) of seeds after either 50 d of burial under 5 cm of soil (hoarded) or exposure on the soil surface (non-hoarded) for seeds of *A. alatum* (a) $N = 11$ depots, *I. deltoidea* (b) $N = 10$ depots, *S. exorrhiza* (c) $N = 18$ depots, and fruits of *D. panamensis* (d) $N = 10$ depots.

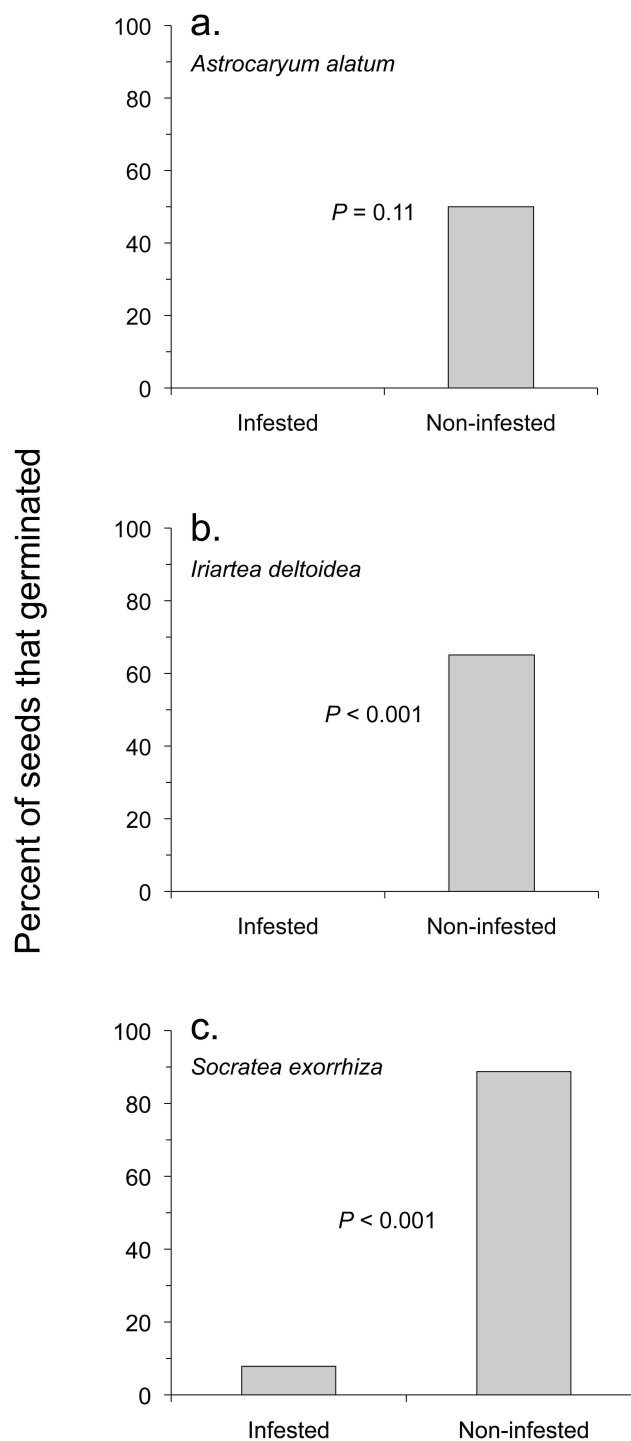


Figure 4.4. Germination success of infested and non-infested *A. alatum* seeds (a, $N_{\text{infested, germinated}} = 0$, $N_{\text{non-infested, germinated}} = 16$, $N_{\text{infested, non-germinated}} = 4$, $N_{\text{non-infested, non-germinated}} = 16$), *I. deltoidea* seeds (b, $N_{\text{infested, germinated}} = 0$, $N_{\text{non-infested, germinated}} = 41$, $N_{\text{infested, non-germinated}} = 59$, $N_{\text{non-infested, non-germinated}} = 22$), and *S. exorrhiza* seeds (c, $N_{\text{infested, germinated}} = 4$, $N_{\text{non-infested, germinated}} = 63$, $N_{\text{infested, non-germinated}} = 47$, $N_{\text{non-infested, non-germinated}} = 8$) after 120 d. All infestation was caused by beetles (*Coccotrypes* sp.).

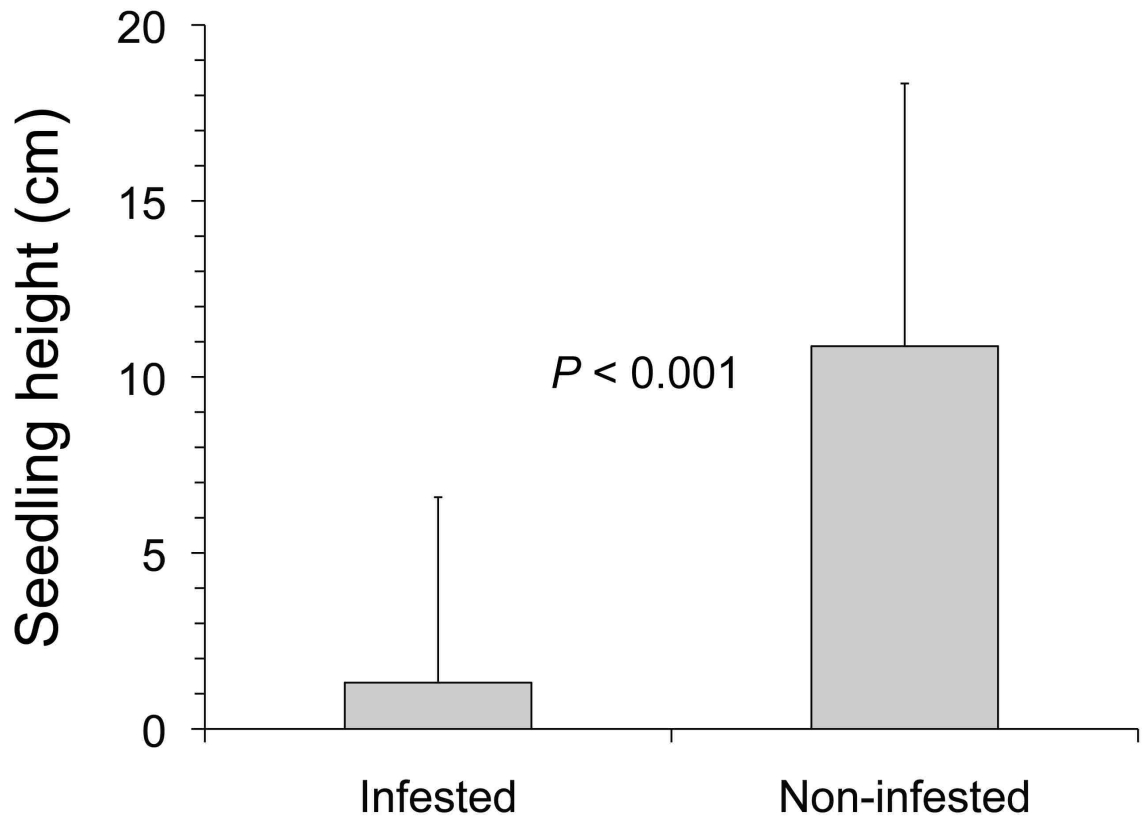


Figure 4.5. Mean heights (+ 1 SD) of *S. exorrhiza* seedlings produced from seeds infested by *Coccotrypes* beetles or non-infested seeds. Seedlings were measured after 120 d of growth in a shade house under natural light and rainfall conditions. $N_{\text{infested}} = 51$ seedlings, $N_{\text{non-infested}} = 71$ seedlings.

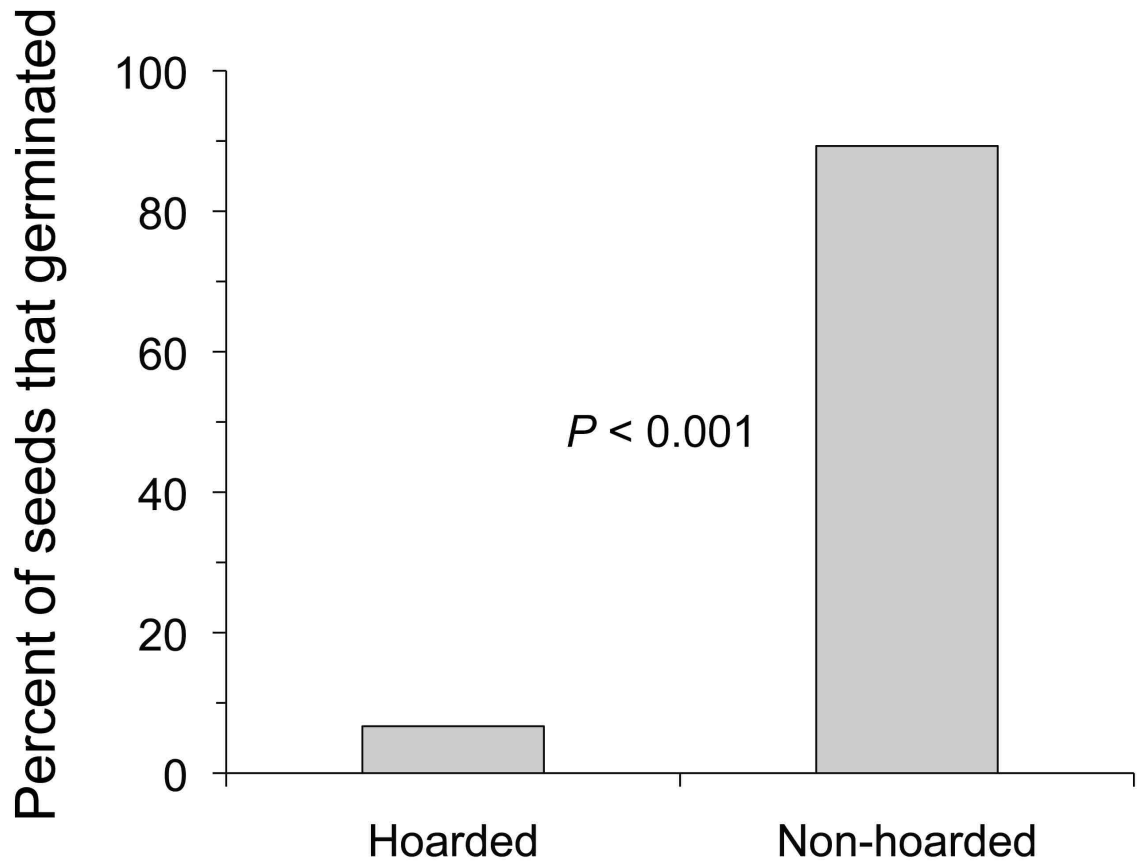


Figure 4.6. Germination success of hoarded and non-hoarded *D. panamensis* fruits after either 50 d of burial under 5 cm of soil (hoarded) or exposure on the soil surface (non-hoarded). $N_{\text{hoarded, germinated}} = 2$, $N_{\text{non-hoarded, germinated}} = 25$, $N_{\text{hoarded, non-germinated}} = 28$, $N_{\text{non-hoarded, non-germinated}} = 3$.

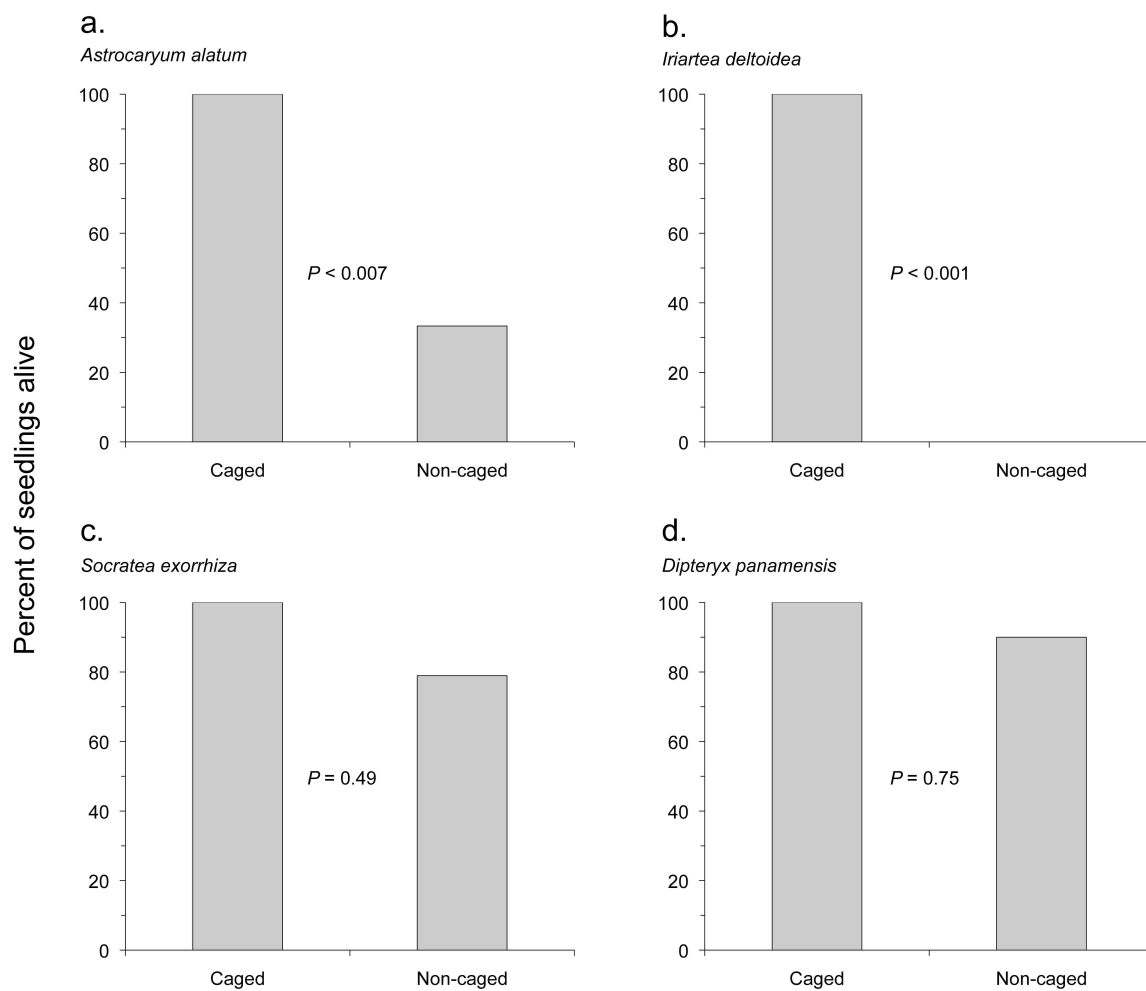


Figure 4.7. Percent of caged (protected from mammals) and non-caged (accessible to mammals) seedlings alive after 140 d in the forest: *A. alatum* (a) $N = 18$ pairs, *I. deltoidea* (b) $N = 17$ pairs, *S. exorrhiza* (c) $N = 19$ pairs, and *D. panamensis* (d) $N = 20$ pairs.

CHAPTER 5

Conclusions

Most interactions between terrestrial mammal granivores and large seeds within La Selva affected plants negatively through seed predation. Previous studies have assumed that seed-eating mammals positively affect plants and seed survival by dispersing seeds, however, in La Selva, I observed very few dispersal and hoarding events. Despite seed predation, terrestrial mammals sometimes provide high quality seed dispersal services to some seeds, greatly improving the probability of the dispersed seeds germinating, growing into seedlings, and potentially contributing to future plant generations. However, dispersal in La Selva was rare, and of dispersed seeds, most were eventually consumed and killed by either peccaries or agoutis. Potentially, seeds scatter-hoarded by agoutis, though uncommon in La Selva, may disproportionately benefit from burial by escaping seed predation by vertebrates and invertebrates to potentially germinate and grow, depending on the hoarded seed species. The main conclusions of my dissertation research are:

- Terrestrial mammal frugivores are abundant within the La Selva rain forest and peccaries are up to six times more abundant than agoutis.
- Due to their high abundances, peccaries encounter and handle seeds on the forest floor before agoutis can find them.
- Peccaries consume and kill most non-defended and chemically-defended seeds within 36 days.

- Physically-defended seeds with non-dehiscing, hard endocarps escape peccary predation and can be dispersed by peccaries via expectoration.
- Agoutis consume non-defended and physically-defended seeds, but cannot eat chemically-defended seeds, however the overall proportions of seeds destroyed by agoutis within 36 days is low.
- Peccaries and agoutis do not differ in seed dispersal distances and thus are equally effective dispersers with regard to seed dispersal distance.
- The chemical defenses possessed by *Mucuna holtonii* seeds (high concentrations of L-dopa) prevent seed predation by rodents, but not predation by peccaries because peccaries can digest toxic materials via pregastric fermentation.
- The coat of *M. holtonii* seeds effectively provides structural protection against seed attack and endosperm excavation by *Sericomyrmex amabilis* ants.
- *Mucuna holtonii* seed endosperm removal by *S. amabilis* ants negatively affects seed germination success and resultant seedling biomass production.
- For large seeds, scatter-hoarding by agoutis greatly enhances seed survival and escape from vertebrate and invertebrate seed predators.
- For large palm seeds, *Coccotrypes* beetles infest most non-hoarded seeds, removing seed endosperm, and hindering seed germination success and consequent seedling growth.

- *Dipteryx panamensis* fruits, overall, did not benefit from hoarding by agoutis because hoarded fruits were unlikely to germinate underneath the soil surface.
- *Astrocaryum alatum* and *Iriarteia deltoidea* seedlings exposed to terrestrial mammals suffered high mortality after five months.
- All seedlings killed by granivorous mammals died from seed removal rather than from herbivory or trampling.

My results show that terrestrial mammals greatly affect the seed and seedling stages of plant life cycles in La Selva. The overall effects that peccaries and agoutis have on seeds will likely influence future forest composition and structure.

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