

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

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(Accepted 8 January 2010)

Abstract: To prevent seed losses from predation, plants have developed protective strategies. Seeds may utilize chemical or structural defences to deter predators. *Mucuna holtonii* (Fabaceae) has large seeds containing a toxic amino acid, L-dopa, and covered with a hard seed coat. Our study assessed the effectiveness of chemical and mechanical seed defences against vertebrate and invertebrate seed predators within Estación Biológica La Selva, 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 the collared peccary (*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 defence against mammals that possess foregut fermentation. The seed coat of *M. holtonii* is an effective structural defence against invertebrate seed predators, preventing endosperm removal and enhancing seedling survival.

Key Words: camera traps, L-dopa, *Pecari tajacu*, seed predation, *Sericomyrmex amabilis*

INTRODUCTION

Seed predation by vertebrates and invertebrates is a major factor limiting the colonization, establishment and growth of plant populations (Forget *et al.* 1999, Howe & Smallwood 1982, Janzen 1971a, b; Schupp 1988). 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 defences that render seeds toxic or inedible to seed-eating animals (Bell 1984, Bodmer 1991, Dirzo & Dominguez 1986, Janzen 1971b, Kiltie 1982).

In tropical rain forests, seeds of several genera in the Fabaceae are chemically defended (Janzen *et al.* 1986, McKenna & McKenna 2006). One classic example of a legume with chemically defended fruits is *Mucuna*, a pantropical genus of lianas comprised of 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 & Janardhanan 2000). Therefore, the seeds of *Mucuna* may serve as a nutritious food resource to granivores that can overcome the seeds' chemical anti-predation defences (Emenalom *et al.* 2004, Udedibie & Carlini 1998a, b).

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

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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, we have observed terrestrial mammals investigating and manipulating these seeds at Estación Biológica La Selva, a tropical rain forest in Costa Rica.

Previous studies have focused only on the function of *M. holtonii* chemical defences 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 defences prevent seed predation. The high toxicity of *M. holtonii* seeds should prevent predation by vertebrate seed predators that cannot overcome the seeds' L-dopa defences. 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 defences in *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 of seeds partially consumed by invertebrate seed predators.

METHODS

Study site

This study was conducted from December 2007 to July 2008 at Estación Biológica La Selva (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 comprised of 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 & Scherry 1980). The pendulous infructescences of *M. holtonii* are comprised of 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 & 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 drilled 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 aluminium 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 \pm 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 (Garcia-Robledo & 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 defence 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 per cent of endosperm removed by invertebrates from each seed. For each depot, we compared the mean per cent 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 \pm 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 after 6 d (Figure 1). We

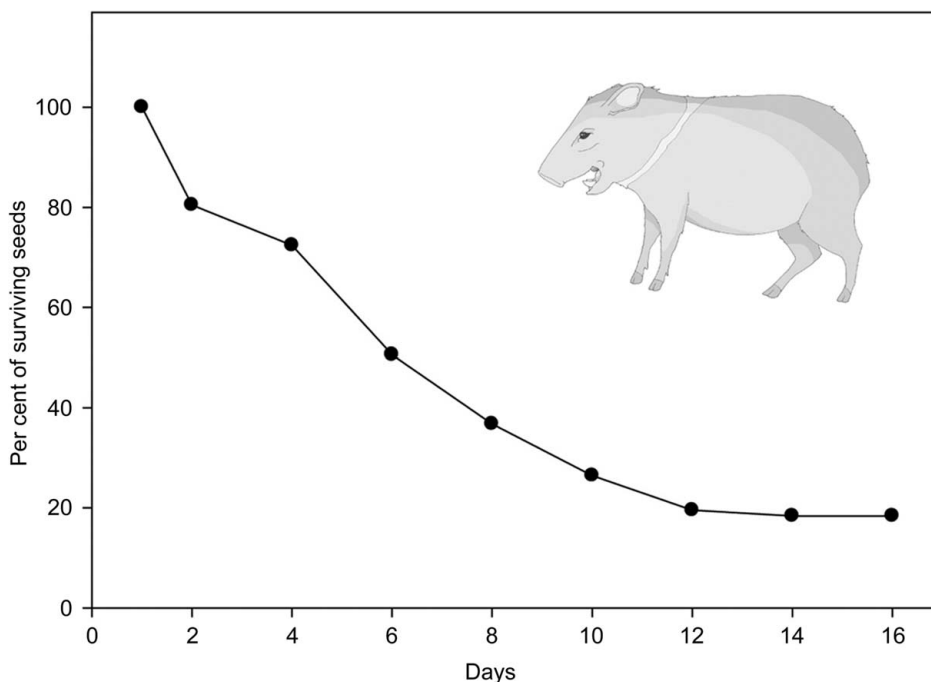


Figure 1. Per cent survival of *Mucuna holtonii* seeds exposed to mammal seed predators over 16 d. Total number of seeds = 90 (six seeds per independent depot).

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 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 the collared peccary (*Pecari tajacu*, Artiodactyla). Most of the seeds removed by peccaries were eaten (98.6%, Figure 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 defence 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 (Figure 2).

DISCUSSION

Pre-dispersal seed predation by insects and fungal infection are leading causes of seed death among many

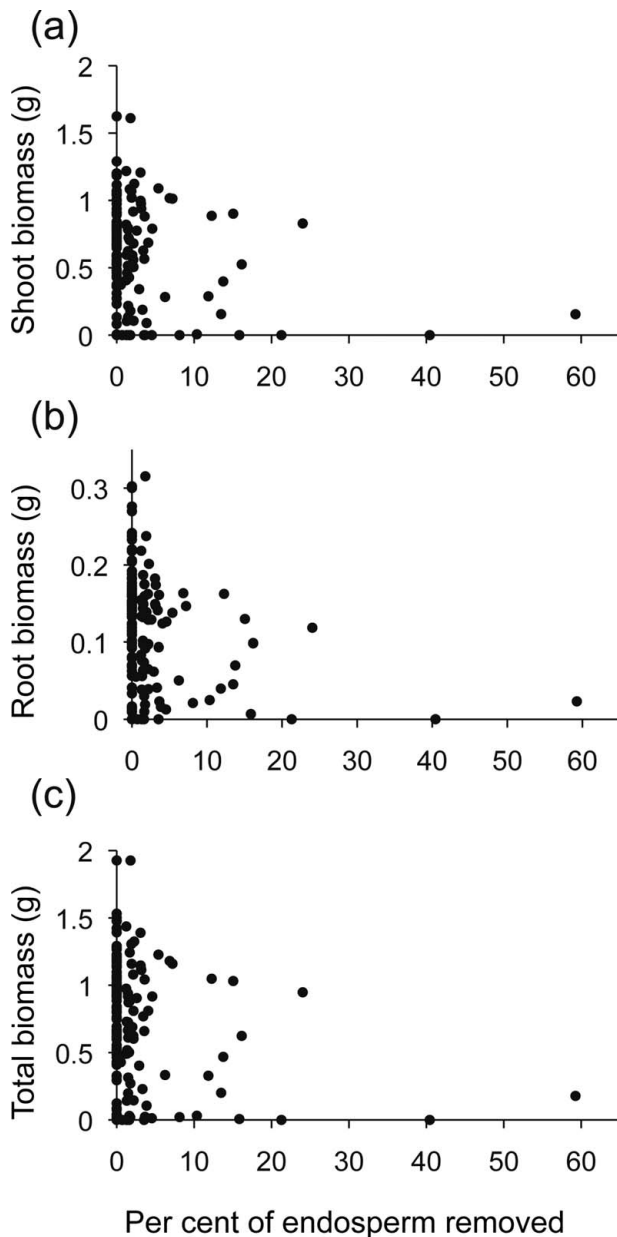


Figure 2. Shoot (a), root (b), and total (c) biomass production (dry weights) of *Mucuna holtonii* seedlings from seeds with different amounts of endosperm removed by *Sericomyrmex amabilis* ants. Linear regressions performed on data after Box-Cox transformations, $N = 143$ plants. Shoot: $\lambda = 0.82$, $F = 5.54$, $df = 1$, $r^2 = 0.031$, $P = 0.02$; root: $\lambda = -1.22$, $F = 7.92$, $df = 1$, $r^2 = 0.046$, $P = 0.0056$; total: $\lambda = 0.78$, $F = 6.05$, $df = 1$, $r^2 = 0.034$, $P = 0.015$.

plant species (Crawley 1992, Green & Palmblad 1975, 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 positively affect seeds of many plant species through seed dispersal, or negatively impact seeds 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 & Brown 1983, Elston *et al.* 2005, Nogueira 2005, Olmo 1993). 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 & 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 defence 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 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 defences of *M. holtonii* do not offer complete immunity against vertebrates as previous studies have reported (Bell & 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 defences provided by the seed coat can also prevent post-dispersal seed predation by invertebrates. Both chemical and mechanical defences are fundamental to the survival and propagation of *M. holtonii* throughout tropical rain forests.

ACKNOWLEDGEMENTS

The authors thank the staff of Estación Biológica La Selva, especially J. Hurtado (TEAM Network, Conservation International) for use of camera traps, D. Brenes (Organization for Tropical Studies) and K. Mora (TEAM Network, Conservation International) for identification of arthropods. Funding for this project was provided by the Organization for Tropical Studies (Emily P. Foster Fund and Rowe Family Fellowship to E. K. Kuprewicz), University of Miami Center for Latin American Studies Grant to E. K. Kuprewicz, and University of Miami Curtis Plant Sciences Scholarships to E. K. Kuprewicz and C. García-Robledo.

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