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Pre-dispersal seed predators and fungi differ in their effect on *Luehea seemannii* capsule development, seed germination, and dormancy across two Panamanian forests

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ABSTRACT

Pre-dispersal seed predation can greatly reduce crop size affecting recruitment success. In addition, non-fatal damage by seed predators may allow infection by fungi responsible for post-dispersal seed losses. The objectives of this study were (1) to quantify pre-dispersal seed predation and fungal infection in a Neotropical tree species, *Luehea seemannii*, that produces dehiscent fruits and wind-dispersed seeds, and (2) to link pre-dispersal effects on seed quality to seed survival in the soil. To examine how seed predators and fungi influence seed losses, mesh exclosures, fungicide, and the combination of both treatments were applied to separate branches in the canopy of trees in Gamboa and Parque Natural Metropolitano (PNM), Panama. To determine if treatments affect seed viability and survival in the soil, half of the seeds collected from each treatment were buried for 4 weeks in forest soils and subsequently allowed to germinate before and after the breaking of dormancy. Overall, 24 percent of developing fruit were lost to insect attack. In contrast, fungi infected only 3 percent of seeds at the pre-dispersal stage. For seeds germinated directly after collection, fungicide significantly increased germination in the wetter site (Gamboa) but decreased germination in the drier site (PNM). The pre-dispersal insect exclosure treatment increased the fraction of seeds that remained dormant after burial in the soil. This result suggests that exposure to insect predators may cause physical damage to seeds that results in the loss of physical dormancy but does not necessarily increase the susceptibility of seeds to pathogen attack in the soil.

Abstract in Spanish is available with online material.

Key words: fungal infection; physical dormancy; predator exclusion experiment; seed burial experiment; seed predation; Tiliaceae.

Pre-dispersal seed predation is a major cause of seed mortality occurring when either immature or mature seeds are attacked on the parent plant (Janzen 1969, 1971, Sanders & Snow 1978, Chambers & Mamcmahon 1994, Zhang *et al.* 1997). Pre-dispersal seed predators are often insects (Janzen 1971, Auld 1991, Chidumayo 1997, Zhang *et al.* 1997, Auld & Denham 2001, De Figueiredo *et al.* 2008, Raimúndez-Urrutta 2008, Pickering 2009, Amri 2014, Delerue *et al.* 2014, Van Klinken & White 2014), but also include vertebrates such as monkeys (Peres 1991, Agetsuma & Noma 1995), mice (Fedriani & Manzaneda 2005), and parrots (Renton 2001, Bollen & Vanelsacker 2004). Fungal pathogens have also been directly implicated in seed losses at the pre-dispersal stage (Sanders & Snow 1978, Travers *et al.* 1998), and may rely on damage from predators to facilitate infection (Willrich *et al.* 2004, Tewsbury *et al.* 2008).

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The consequences of pre-dispersal seed predation can be considered at both population and community levels. At the population level, seed predation limits the colonization ability of plants by reducing seed crop size (Anderson 1988, Greig 1993, Delerue *et al.* 2014), and consequently, both the density and spatial extent of seed dispersal (Dirzo & Domínguez 1986). At the community level, reduced dispersal capacity may reduce opportunities for competitive exclusion, facilitating species coexistence (Hurtt & Pacala 1995). Seed predators may also have subtler effects by reducing the vigor of seedlings derived from infested seeds (Dalling *et al.* 1997a) or by increasing the susceptibility of seeds to post-dispersal predation or infection from pathogens. For example, insect damage may predispose seeds to fungal infection in the soil (Mills 1983, Kremer & Spencer 1989).

Fungal infection of fruits and/or seeds may also occur before dispersal. In the crown, seeds may be infected by airborne (Moussart *et al.* 1998) or pollinator/predator vectored fungi (Janzen 1971, Mills 1983, Travers *et al.* 1998, Lara & Ornelas 2003). In cotton (*Gossypium hirsutum* L), boll-rotting fungal

pathogens (*Diplodia* spp. and *Fusarium* spp.) are associated with green stink bugs and reduce germination of seeds from harvestable fruit (Willrich *et al.* 2004, Bommireddy *et al.* 2007). Rust pathogens also reduce reproductive output of trees in temperate and tropical regions (Travers *et al.* 1998, Tillman-Sutela *et al.* 2004). Seed deterioration from fungal infection in the crown has been documented in various crop species (*e.g.*, Mills 1983, Willrich *et al.* 2004, Bommireddy *et al.* 2007). However, the effects of pre-dispersal fungal infection on seed production in tree species and its post-dispersal consequences have not been well studied (Travers *et al.* 1998).

Pre-dispersal seed losses have been documented for many plant species, particularly herbs and shrubs (*e.g.*, Janzen 1969, Augspurger 1981, Desteven 1981, Louda 1983, Greig 1993, Tomaz *et al.* 2007, Pickering 2009, Delerue *et al.* 2014) and agricultural crops (*e.g.*, Sperens 1997, Gomes *et al.* 2005). However, few studies of pre-dispersal seed predation and fungal attack exist for tropical tree species (Beckman & Muller-Landau 2011), and most of these studies rely on observational methods (Travers *et al.* 1998, Forget *et al.* 1999, Beckman & Muller-Landau 2007). The effect of mortality agents on seed survival has rarely been experimentally measured for tree species because of the difficulty of tracking events that occur high above the ground. In this study, our objectives were (1) to quantify pre-dispersal seed predation and fungal infection in a common pioneer tree species, *Luehea seemannii* Triana and Planch (Tiliaceae), that produces dehiscent capsular fruits and wind-dispersed seeds, and (2) to link pre-dispersal effects on seed quality to post-dispersal survival of seeds in the soil. We hypothesized that (1) if pre-dispersal seed predators and fungal species are significant sources of seed losses, then excluding them from developing capsules will increase production of intact, mature fruit, and viable seeds. (2) If probing insect predators in the canopy damage seeds providing opportunities for fungal infection in the soil, then we predicted that seeds exposed to seed predators will have greater reductions in germinability after incubation in the soil than seeds that were protected from seed predators.

METHODS

Study species and sites.—*Luehea seemannii* (Tiliaceae) (hereafter referred to as *Luehea*) is a monoecious species common in secondary forest and along forest edges. *Luehea* occurs in lowland forests from southern Mexico to northern South America (Croat 1978). In Panama, *Luehea* is common in secondary forests along the Pacific slope of the continental divide. Trees are 15–30 m tall and up to 125 cm dbh. *Luehea* trees produce axillary or terminal inflorescences (Borchert 1996) in the mid to late dry season (January–March). The fruit, which mature late in the dry season and early in the wet season (late March–July), are elliptical dehiscent capsules 2.5 cm long, 1 cm in diameter, and have five deep grooves. Once mature, the distal portion of the capsule opens to release on average 40 (SD = 11.7), 1.9-mg-wind-dispersed seeds. Dispersal units are samaras 6–10 mm long. The seeds are small, oblong, about 2.5–3 mm long, and 1 mm wide. The embryo is straight and fleshy (Fournier 2002). Fecundity of *Luehea*, estimated from seed trap collections, is high (240 seeds/cm^2 basal area); median dispersal distance estimated from seed trap data is 8 m (Dalling *et al.* 2002) and from genotype data 20–26 m (Jones *et al.* 2004). Seeds are common in the soil seed bank and persist in understory sites for 1–2 yr (Dalling *et al.* 1997b). A fraction of fresh seeds are initially dormant, requiring immersion in hot water (70–80°C) to trigger germination (Acuña & Garwood 1987, Dalling *et al.* 1997b, Sautu *et al.* 2007).

Experiments to assess the importance of pre-dispersal seed predation on *Luehea* were carried out at two sites from March 2008 to August 2008. Sites were located in Parque Natural Metropolitano (PNM) (8°58′ N, 79°34′ W) in Panama City and Gamboa, 30 km north of Panama City. In PNM, we used a construction crane operated by the Smithsonian Tropical Research Institute (STRI) to access the canopy. The crane is 42 m tall and can access tree crowns within a 48 m radius of the tower. The forest at PNM is a 75- to 150-yr-old-second-growth stand with tree heights up to 40 m (Kitajima *et al.* 1997). Canopy dominants include *Luehea* and *Anacardium excelsum*. PNM receives an average of 1850 mm of rain per year. The dry season is approximately from mid-December to the end of April (Environmental Science Program, STRI 2008). The annual mean temperature is 27°C. A second population of *Luehea* was located in old second-growth forest in Gamboa (9°7′ N, 79° 42′ W), 20 km north of PNM. At Gamboa, fruits on low-hanging branches along the forest edge were accessible by a ladder. Climate is similar at Gamboa to PNM; however, annual rainfall is higher at 2131 mm (Meteorological and Hydrological Branch, Panama Canal Authority 2008).

Observation of capsules and seeds.—To estimate capsule damage without the influence of our experiments, we recorded damage of capsules and seeds on shoots outside of our experimental treatments at PNM, referred to as 'exposed' shoots. We tagged capsules from 17 exposed shoots (9–33 capsules per shoot) on branches adjacent to experimental treatments at the time the experiment was set up. From these exposed shoots, we recorded the number of (1) intact, fully mature capsules containing intact seeds and (2) capsules with evidence of either insect chewing or insect exit holes.

To determine whether damaged capsules were able to release seeds, 35 damaged capsules were chosen at random from three trees at PNM and enclosed in small mesh bags during the experiment. The three trees were reachable by crane and were the same as those used in the canopy experiment described below. From these damaged capsules, we recorded the number of intact seeds released. At the end of the experiment, we collected damaged capsules and recorded the number of seeds not released from capsules in the following categories (1) intact seeds (shed vs. non-shed from the capsules), (2) seeds damaged by insects in which whole or part of the dispersal unit was eaten, and (3) seeds with fungal hyphae covering the surface.

Canopy experiment to exclude insect predators and fungi.—To examine how seed predation and fungal infection might affect

seed viability, we applied six treatments to groups (shoots) of developing capsules on sections of *Luehea* branches. Treatments were (1) control with no manipulation (hereafter referred to as control), (2) mesh exclosure, (3) sham exclosure, (4) fungicide, (5) water addition, and (6) fungicide and mesh exclosure. Mesh exclosures were made from fine nylon mesh (0.2-mm mesh size) to exclude insects. A square mesh cloth was folded to form a cone of approximate 2 L volume, which enclosed an average of 19 (SD = 6.5) developing capsules. In the sham exclosure treatment, the mesh was cut lengthwise to allow predators access; sham exclosures were used to inspect for potential physical effects of mesh on fruit development. The effect of sham exclosures was compared with the control treatment.

The fungicide Captan 48.9 percent Wettable Powder (Micro Flo LLC, Memphis, TN) was used to treat *Luehea* capsules. Captan (N-Trichloromethylthio-4-cyclohexene-1, 2-dicarboximide) is a non-systemic fungicide that protects against a wide range of fungi (*e.g.*, *Fusarium* and *Rhizoctonia*) as well as oomycetes (*e.g.*, *Phytophthora*, *Pythium*) (Office of Pesticide Program 1999). It is reported to be particularly effective against seed-rotting fungi (Wainwright & Pugh 1975, Jeffs 1986) without an observable effect on seed germination (Gallery *et al.* 2007). Fungicide was applied by immersing shoots in 1 L of a 10 g/l Captan solution for 30 s. The water addition treatment was used to inspect for the effect of immersing capsules in aqueous solution. The effect of water addition was compared with the control treatment. Fungicide or water was applied every 2 weeks until seed collection was finished (seed collection method: see Appendix S1). For the fungicide and exclosure treatment, shoots were first dipped in the fungicide mixture before covering with an insect exclosure. Subsequently, fungicide was applied without removing the mesh exclosures.

The number of experimental trees and shoots at PNM and Gamboa was limited by the accessibility of treecrowns. At PNM, four trees were used in the experiment; three trees were accessible from the construction crane and one tree with low branches could be reached from the ground. At Gamboa, the canopies of three trees along a forest edge were accessible from a step-ladder. At each tree, the six treatments were assigned randomly to experimental shoots arrayed along the same branch. When space did not permit all treatments to be accommodated on the same branch, then adjacent branches <2 m apart were used. In total, 23 branches were used in the experiment. Experimental shoots, consisting of similar branch lengths, differed in the number of developing capsules (average of 19 capsules SD = 6.5).

Each *Luehea* individual initiated flowering asynchronously in early February so that by mid-February branches contained flowers and some immature fruits. Most of the flowers were fertilized over 2-week period toward the end of February. Trees at the different sites developed capsules at different times. Treatments were applied at Gamboa from 3 to 6 March 2008. At PNM, treatments were set up from 6 to 20 March 2008. In total, 11 sets of treatments were established at Gamboa and nine sets at PNM. During the experiment, 10 individual shoots were lost at Gamboa, when wind damage snapped branches; therefore, in total, 98 individual shoots survived until seed collection (using mesh bags) was finished (Table S1). Prior to capsule dehiscence, all treatments were enclosed in mesh to collect seeds for germination trials (seed collection method: see Appendix S1). At the end of the experiment, capsules from experimental shoots were visually inspected and categorized as aborted, externally damaged with evidence of either insect chewing or insect holes, or intact, fully mature capsules that were outwardly healthy.

Seed germination and dormancy.—In total, 81 of 98 experimental shoots shed sufficient seeds for use in germination and burial experiments (Table S1). When possible, seeds from each experimental shoot were divided into four lots of 15 seeds each. The first two lots collected from an experimental shoot were used to test initial seed germination, and the remaining seed lots from that shoot were used in the burial experiment. Seeds from each lot were placed on tissue-paper-lined Petri dishes and moistened with tap water. In total, 158 lots were used in the initial germination test carried out under 30 percent full sun in a screened growing house in Gamboa. Seed germination was recorded over 8 weeks from 5 July to 22 August 2008. Germination was scored weekly as the emergence of a 2-mm-long radicle and/or green cotyledons. Seeds were removed after germination. Seeds infected by fungi were removed and placed individually in additional tissue-paper-lined Petri dishes to prevent spread to uninfected seeds. Since some fresh *Luehea* seeds exhibit initial physical dormancy (Sautu *et al.* 2006, 2007), ungerminated seeds remaining in Petri dishes after 6 weeks were submerged for 2 minutes in 80°C water, a dormancy-breaking treatment (Acuña & Garwood 1987). Germination was recorded for an additional 2 weeks. Seeds that germinated during the 2 weeks following the dormancy-breaking treatment were classified as dormant. After the additional 2 weeks, germination trials were terminated and the non-germinated seeds were classified as non-viable.

Seed burial experiment.—After testing initial viability, remaining lots of 15 seeds were placed in individual nylon mesh bags (0.5-mm mesh size) together with 10 g of autoclave-sterilized forest soil. Mesh bags retained the seeds but were permeable to fungi and small invertebrates (Gallery 2007). In total, 143 bags were buried at random 30 cm apart and at 3 cm depth in a single 3×3 m^2 common garden beneath the forest canopy in Soberania National Park, Gamboa. As *Luehea* seeds lose viability after short periods of burial (50% reduction within 4 months) (Dalling *et al.* 1997b), and fungal infection of susceptible seeds is likely to occur rapidly (Gallery *et al.* 2007), seeds were recovered after only 4 weeks in the soil in mid-July 2008. The contents of individual mesh bags placed in Petri dishes and germination and seed dormancy were scored as above.

Data analysis.—We used generalized linear mixed models (GLMMs) to analyze the influence of seed predators and fungal pathogens on capsule maturation and seed germination. Natural enemy exclusion treatments and site were included as fixed effects. The fixed factors include water addition, sham exclosure, fungicide, and mesh exclosures each with two levels (control and

treatment) as well as site with two levels (Gamboa and PNM). We included two-way interactions between water addition and site as well as sham exclosures and site, and two- and three-way interactions among fungicide, mesh exclosures, and site. For the analysis, the control and Gamboa serve as reference levels for factors. Hence, the intercept of the GLMM is the mean (log of odds ratio) of the control in Gamboa, and coefficient estimates for main effects are differences from this mean. If an interaction between treatment and site is significant, we also report the coefficient estimate and standard error for the difference between the treatment and the control in PNM. To account for spatial autocorrelation among capsules and seeds within shoots and shoots within trees, shoots nested within tree were included as random effects. For the analysis of the proportion of intact, mature capsules, capsules were considered the experimental unit. The proportion of germinating seeds and proportion of dormant seeds were analyzed separately for fresh seeds collected from the canopy and buried seeds recovered from the soil. The proportions of seeds that germinated were considered following the dormancy-breaking treatment with hot water. In these analyses, seeds were considered the experimental unit. The proportion of intact, mature capsules, germinating seeds, and dormant seeds were analyzed with binomial errors. We used the Laplace approximation of likelihoods to estimate parameters of fixed and random effects using the *lme4* package in R (Bates *et al.* 2015, R Development Core Team 2015).

RESULTS

Observed variation in capsule and seed losses.—From the 17 exposed shoots (without treatment application) in PNM, we found that on average 76 percent (± 4.8 (SE), range: 31–100%) of capsules were intact, fully mature, and contained intact seeds. The remaining 24 percent (± 4.8 [SE]) showed an insect exit hole or insect chewing scars.

From the 35 damaged capsules, on average, half (50.0% ± 4.4 [SE]) of the seeds collected were themselves damaged (Fig. 1). Most of the remaining seeds (46.2% ± 4.1 [SE]) were intact; however, only 8.9 percent (± 2.9 [SE]) of all intact seeds in a damaged capsule were released from the capsules (Fig. 1). On average, 3.8 percent (± 2.3 [SE]) of seeds in a damaged capsule were infected by fungi (Fig. 1) and showed visible fungal hyphae; infected seeds were found among capsules that matured in the wet season (late June–July).

Most seed damage could be attributed to an unidentified, 3–4 mm long Eucnemidae beetle; the only insect to emerge from capsules during capsule storage. The 2-mm diameter exit holes produced by this beetle were similar to those observed on capsules in our experimental treatments. A damaged capsule typically had a single exit hole; damage to seeds within the capsule was often limited to few locules of the fruit.

Effects of insects and fungi on capsule damage and maturation.—In the canopy exclusion experiment, 0–100 percent (mean 31.0 ± 2.3% SE) of capsules per shoot failed to mature due to abortion or insect damage: 0–90.5 percent (mean 23.6 ± 2.1% SE) of capsules per shoot were aborted and 0–69.2 percent (mean 9.8 ± 1.4% SE) of capsules had evidence of insect damage during development.

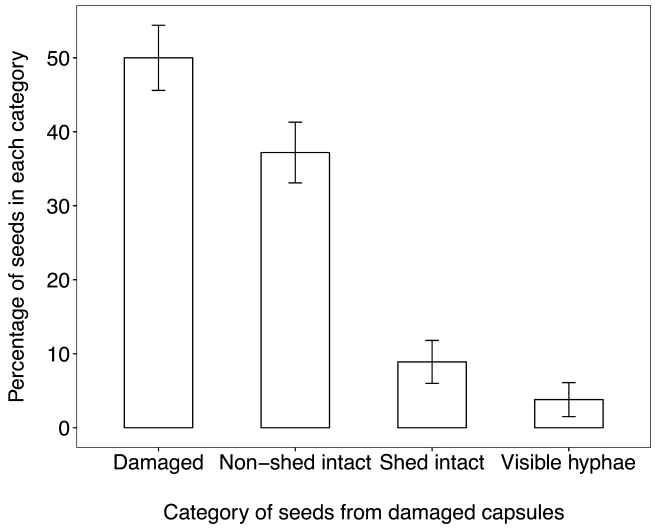

FIGURE 1. The mean percentage of seeds in four seed categories found in damaged capsules (N = 35 capsules). The categories include damaged seeds, non-shed intact seeds, shed intact seeds, and seeds with visible fungal hyphae. Error bars represent ± 1 SE.

In the GLMM, there were no significant differences in the proportion of intact, mature capsules that developed in the controls between PNM and Gamboa (Figs. 2A, 3A and S1; Table S2). The proportion of mature capsules varied significantly in response to fungicide across sites as indicated by a significant negative interaction between site and the fungicide treatment (coefficient estimate ± SE = −2.42 ± 0.68, z = −3.57, P < 0.001). At Gamboa, the proportion of mature capsules significantly increased by 30 percent in the fungicide treatment compared with controls (coefficient estimate ± SE = 1.23 ± 0.45, z = 2.74, P < 0.01), while in PNM, they significantly decreased by 34 percent in the fungicide treatment compared with controls (coefficient estimate ± SE = −1.20 ± 0.51, P < 0.05).

The proportion of mature capsules varied significantly in response to sham exclosures across sites as indicated by a significant negative interaction between site and sham exclosures (coefficient estimate ± SE = −1.72 ± 0.56, z = −3.09, P < 0.01). In Gamboa, the proportion of mature capsules significantly increased by 22 percent in the sham exclosures compared with the controls (coefficient estimate ± SE = 0.82 ± 0.39, z = 2.07, P < 0.05), and in PNM, they significantly decreased by 25 percent in the sham exclosures compared with the controls (coefficient estimate ± SE = −0.90 ± 0.39, z = −2.29, P < 0.05). There was also a marginally significant three-way interaction among insect exclusion, fungicide application, and site (coefficient estimate 1.87 ± 0.98, z = 1.91, P = 0.06) as the effect of the fungicide treatment across sites differed from that of the exclosure treatment and the application of both insect exclosures and

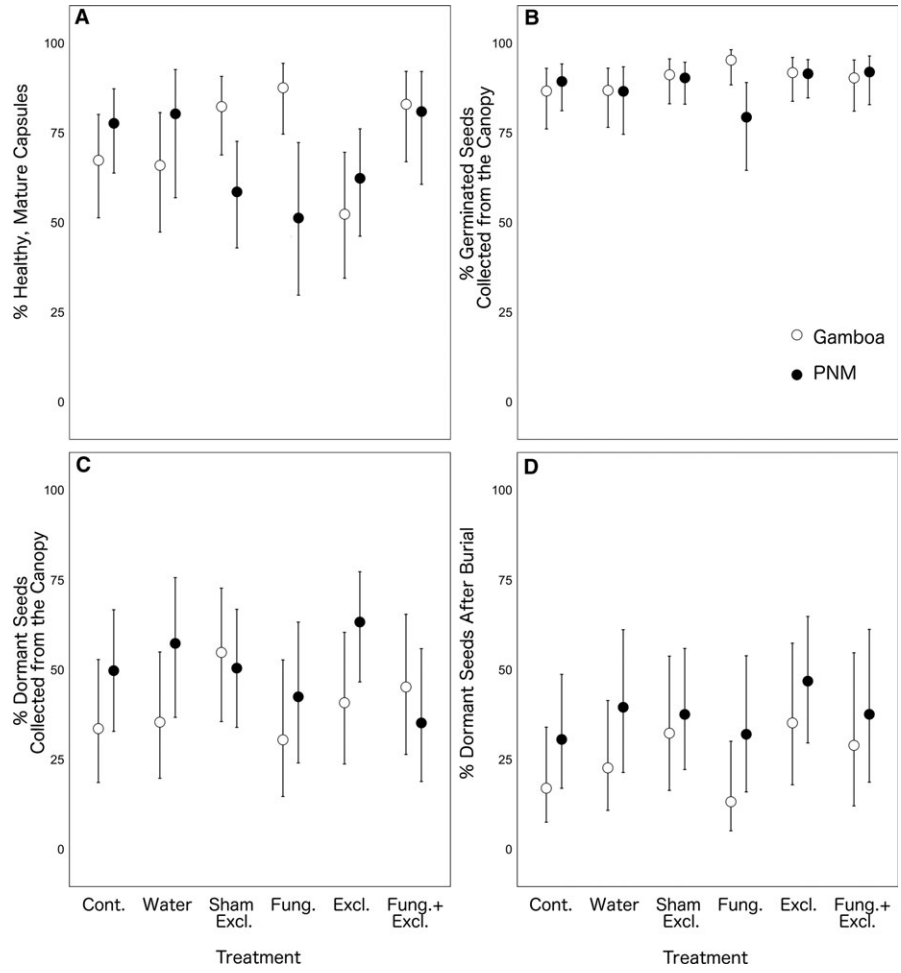

FIGURE 2. The effect of natural enemy exclusion treatments on the probability of *Luehea* (A) capsule maturation, (B) seed germination, (C) dormancy of canopy-collected seeds, and (D) seed dormancy after burial. Symbols and error bars represent the means of responses ± 2 SE (on the original scale), estimated from a generalized linear mixed model using capsules as replicates for each treatment in Gamboa (open circles) and Parque Natural Metropolitano (PNM) (filled circles). Cont. = control, unmanipulated capsules; Water = water addition; Sham Excl. = mesh exclosure with openings to allow insect access; Fung. = fungicide addition; Excl. = mesh insect exclosure; Fung.+ Excl. = fungicide and exclosure.

fungicide (Figs. 2A and 3A). None of the other treatments nor their interactions with site were significant (Table S2). There was large variation in the random effects of capsule development among shoots nested within trees (standard deviation, SD = 0.60) and among trees (SD = 0.35; Table S2).

EFFECTS OF INSECTS AND FUNGI ON INITIAL SEED GERMINATION AND DORMANCY.—The observed initial viability of *Luehea* seeds was high (88%), but varied widely (Fig. 2B). In the GLMM, the proportion of fresh seeds that germinated in controls did not vary between sites (Figs. 2B, 3B and S2; Table S3). The proportion of seeds that germinated varied significantly in response to fungicide across sites as indicated by a significant interaction between site and fungicide treatment (coefficient estimate ± SE = −1.91 ± 0.68, $z = -2.80$, $P < 0.01$). At Gamboa, the fungicide treatment significantly increased germination of fresh seeds by 10 percent compared with controls (coefficient estimate = 1.13 ± 0.53, $z = 2.16$, $P < 0.05$), whereas in PNM, the fungicide reduced germination by 11 percent compared with controls and was marginally significant (coefficient estimate −0.78 ± 0.43, $z = -1.79$, $P = 0.07$). There was also a marginally significant two-way interaction between exclosure and fungicide treatments (coefficient estimate −1.31 ± 0.70, $z = -1.88$, $P = 0.06$) and a three-way interaction among the exclosure, fungicide application, and site (coefficient estimate 2.15 ± 0.95, $z = 2.27$, $P < 0.05$) as the effect of fungicide treatment across sites differed from that of the exclosure treatment and the application of both insect exclosures and fungicide (Figs. 2B and 3B). All other treatments and interactions were not significant (Table S3). There was large variation in the random effects of initial germination among shoots nested within trees (SD = 0.55) and among trees (SD = 0.33; Table S3).

At Gamboa, fungal infection was significantly higher in untreated seeds (3%) compared with fungicide-treated seeds

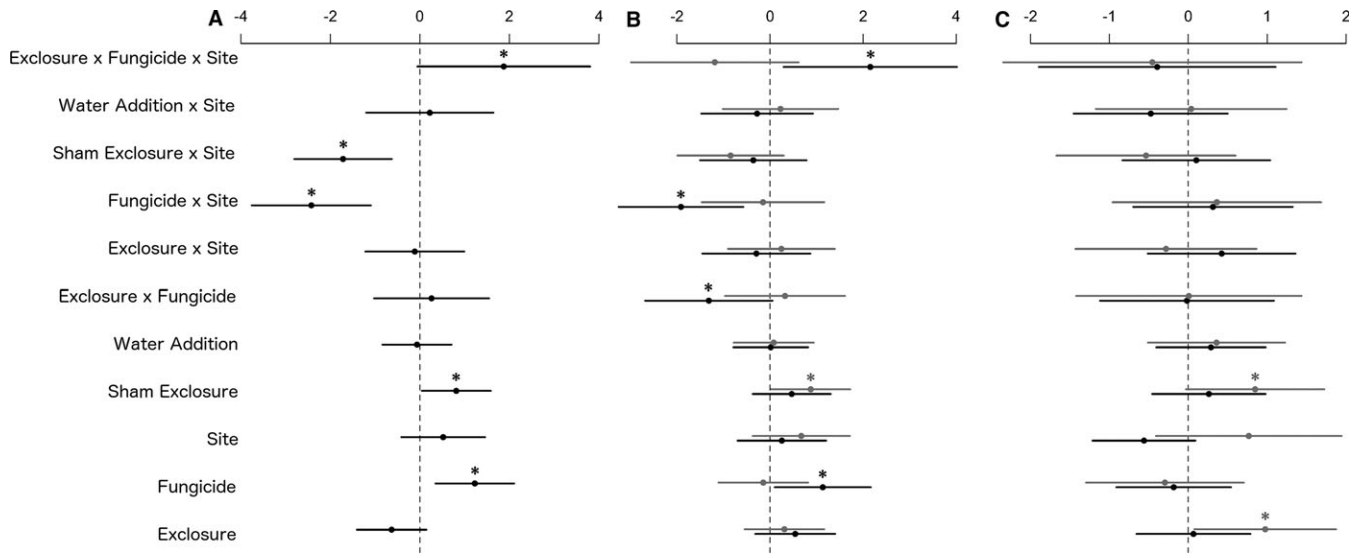

FIGURE 3. Coefficient estimates (±2 SE) of generalized linear mixed models representing differences from the mean response (log of odds ratio) in the control in Gamboa for (A) capsule maturation, (B) seed germination (black) and dormancy (gray) of canopy-collected seeds, and (C) seed germination (black) and dormancy (gray) after burial. Asterisks indicate significant effects of the treatments at a significance level of $\alpha = 0.05$. In Fig. 3B and C, black asterisks represent the significant effects for seed germination, while gray asterisks show significant effects for seed dormancy. For more details of results, see Tables S2–S6.

(0.3%) (Binomial test, chi-square = 7.2, df = 1, $P < 0.01$). The incidence of fungal infection tended to be lower at PNM and was not significantly different between untreated (2%) and treated seeds (0.2%) (Binomial test, chi-square = 3.2, df = 1, $P = 0.07$).

We found that 44.9 ± 2.3 percent of fresh seeds collected from controls in the canopy were dormant. In the GLMM, these did not vary between sites (Figs. 2C, 3B and S3; Table S4). We found that sham exclosures in Gamboa significantly increased dormancy by 63 percent compared with controls (coefficient estimate ± SE = 0.88 ± 0.43, $z = 2.03$, $P < 0.04$). There was large variation in the random effects of initial dormancy among shoots nested within trees (SD = 0.70) and among trees (SD = 0.42; Table S4).

Effects of insects and fungi on post-burial seed germination and dormancy.—After burial in soil for 4 weeks, we observed a 23 percent reduction in seed germination. In the GLMM, the treatments did not affect germination (Fig. 3C, Table S5). There was no difference in the proportion of dormant seeds collected from controls following burial across sites (Figs. 2D, 3C and S4; Table S6). In Gamboa, insect exclosures significantly increased the proportion of seeds that were dormant by 107 percent compared with controls (coefficient estimate = 0.98 ± 0.46, $z = 2.13$, $P = 0.03$), and sham exclosures marginally increased the proportion of seeds that were dormant by 90 percent compared with controls (coefficient estimate = 0.85 ± 0.45, $z = 1.90$, $P = 0.058$). There was large variation among shoots nested within trees in post-burial germination (SD = 0.48) and dormancy (SD = 0.63) and among trees in dormancy (SD = 0.55) but not germination (SD = 0; Table S5–S6).

DISCUSSION

Variation in capsule and seed losses.—Pre-dispersal seed losses are notoriously variable both among and within species occupying the same site (Janzen 1969, Janzen & Vasquez-Yanes 1991, Chidumayo 1997, Auld & Denham 2001, Beckman & Muller-Landau 2011, Amri 2014, Delerue *et al.* 2014). In our study, on average 24 percent of the capsules on exposed shoots were damaged, and these had evidence of pre-dispersal seed predation. However, within a single tree, capsule damage was highly variable among shoots. From direct observation of capsules with visible signs of insect damage, on average half of the seeds were damaged (ranging from 0 to 100%) and 3.8 percent showed visible fungal hyphae (ranging from 0 to 67%).

In the canopy experiment, we found large variation within and among trees in capsule development as well as initial and post-burial germination and dormancy in response to insect seed predators and fungi. For each response, variation among shoots within trees tended to be higher than among trees (standard deviation of random effects, Tables S2–S6). This variation was on the order of magnitude of treatment effects and smaller than the largest treatment effect. SD of random effects relative to the largest treatment effect ranged from 0.25 to 0.86 among shoots within trees and from 0 to 0.62 among trees (Tables S2–S6). This relative variation tended to be smallest for capsule development and initial germination (Tables S2–S6). Similarly high variation in pre-dispersal damage to seeds and fruit has been reported in many studies (*e.g.*, Janzen 1969, Louda 1983, Greig 1993, Crawley & Long 1995, Kolb *et al.* 2007, Beckman & Muller-Landau 2011) ranging from 0 to 100 percent. Key functional traits such as fruit and seed morphology can help explain some of the variation,

both within and among species (Beckman & Muller-Landau 2011).

In addition to capsule losses directly attributable to seed predators, a fraction of capsules (21%) in the control treatments of both study sites failed to mature and were aborted. Mesh bags enclosing capsules were found to contain both damaged and undamaged capsules that were aborted before maturity. Fruit abortion may result from both intrinsic and extrinsic factors, including seed genotype, inbreeding depression, developmental abnormalities, resource availability, and environmental conditions (Stephenson 1981, Ganeshaiah & Shaanker 1988, Kärkkäinen *et al.* 1999, Collevatti *et al.* 2009). Furthermore, the aborted fraction in this study may have been underestimated if flowers and developing capsules were abscised before the first census of developing capsules was made.

Effects of insects on capsule and seed losses.—We observed damage to *Luehea* capsules and seeds by insects on exposed shoots, but did not find significant effects of insect seed predators on capsule development or initial germination and dormancy. Two different types of damage to *Luehea* capsules were observed. At PNM, chewing damage was observed on a few capsules on two trees, possibly attributable to Meliponine (stingless) bees (N. Beckman, pers. obs.). Sap exudation from these damaged capsules prevented the dehiscence and release of seeds. In addition, damage consisted of exit holes made by the adults of an unidentified Eucnemidae beetle that developed within the capsule. A single 3- to 4-mm-long beetle developed within each infested capsule; larvae developed by feeding on multiple seeds, but typically not all locules within the capsule were damaged. Nonetheless, damaged capsules mostly failed to open and therefore dispersed very few seeds. The biology of Eucnemid beetles remains largely unknown (Evans & Hogue 2006). Adult Eucnemidae are usually collected on plant surfaces including trunks and stumps (Muona 2011). Eucnemid larvae have liquid-feeding mouthparts, so their actual diet is difficult to ascertain (Lawrence *et al.* 1995, Muona 2011). We found that insect seed predators not only damage capsules and seeds but can limit the dispersal capacity of intact seeds within damaged capsules. With direct observation of damage capsules, we found about 46 percent of seeds were intact; but only 8.9 percent of the intact seeds were released from the insect-damaged capsules. However, this is an underestimate of effective seed loss as most seeds in damaged capsules were not shed and would likely rot beneath the tree.

Effects of fungi on capsule and seed losses.—Although the magnitude of differences among treatments is small, our findings supported the hypothesis that pre-dispersal fungal infection influences capsule production and seed germination. However, the effect differed between sites for both responses.

Fungi affected capsule production differentially in the two sites (Figs. 2A and 3A). At the pre-dispersal stage, fungal exclusion increased the percentage of mature capsules in *Luehea* individuals from the wetter site (Gamboa), while applying fungicide slightly reduced the percentage of mature capsules in the dryer site (PNM). Differential response to fungal exclusion between localities may arise because of the microclimate during fruit development. The amount of rainfall during the period of canopy experiments (March–April) was 78 and 17 mm for Gamboa and PNM, respectively (Meteorological and Hydrological Branch of the Panama Canal Authority, 2008). For an 11-year record (2003–2013), the rainfall during March and April ranged from 59 to 269 mm in Gamboa and ranged from 17 to 307 mm in PNM (Meteorological and Hydrological Branch of the Panama Canal Authority, 2008). Gamboa experienced wetter conditions than PNM during the time period of the study, and this could negatively affect capsule production because the moist conditions are positively associated with fungal abundance and richness (Timmer *et al.* 2000, Talley *et al.* 2002), and increase the risk of pathogenic fungal infection (Timmer *et al.* 2000, Xu 2003). At the dryer site, PNM, where the fungal loads on capsules were lower, fungicide may have been toxic and negatively affected the development of seeds.

Germination of *Luehea* seeds was also affected by pre-dispersal fungal infection. Consistent with capsule production, we found different responses of seed germination between the two sites. Fungicide treatment increased germination of *Luehea* before burial in the wetter site of Gamboa, but reduced germination in the drier site of PNM. The positive effect of fungicide application was in agreement with a previous study in seven Neotropical species including *Luehea* (Beckman & Muller-Landau 2011). In addition, negative effects of fungicide on seed germination have also been reported in many tree species (*e.g.*, Cox *et al.* 2011, Beckman & Muller-Landau 2011, but see Gallery *et al.* 2007). The negative effect is commonly interpreted as chemical properties of fungicide inhibiting germination (Cox *et al.* 2011). In this study, seeds of *Luehea seemannii* responded both negatively and positively to the fungicide treatment. At PNM, where fungal infection rates were low, fungicide may have had a negative inhibitory effect on germination. At Gamboa, where fungal loads were higher, the positive effect of killing fungi may have outweighed any negative inhibitory effects.

Overall, few fresh seeds collected directly from the canopy showed visible sign of fungal infection; two to three percent of seeds sown in Petri dishes showed sign of mycelial growth. However, the fungal infection in this study is underestimated because fungi can colonize seeds in the canopy without showing visible signs of infection (Mills 1983, Rodriguez *et al.* 2009). In addition, rates of plant fungal infection are sensitive to changes in environmental conditions and may show interannual temporal variation (Gallery 2007, García-Guzmán *et al.* 2016). To determine whether the magnitude of seed losses, and the site differences observed in this study are attributable to weather conditions or to other factors would require replication of the experiment across multiple years. As the study period was relatively dry, higher seed losses might be expected under 'average' conditions.

Effect of pre-dispersal seed predation by insects on post-burial dormancy.—We did not find support for the hypothesis that pre-dispersal damage to seeds increases the susceptibility to

pathogen infection in the soil, as there were no treatment effects on seed germination after burial. However, we did find evidence that pre-dispersal exposure to seed predators affects seed dormancy.

Seed dormancy is widespread among larger-seeded pioneer species (>1 mg seed mass) in lowland forests of Panama (Dalling *et al.* 1997b). In this study, despite the small magnitude of differences among treatments, the exclusion of insect seed predators from *Luehea* fruit at Gamboa increased the fraction of seeds that were dormant after burial in the soil. The increase in the proportion of dormant seeds when protecting seeds from insects was in agreement with a previous study of seven Neotropical species, including *Luehea* (Beckman & Muller-Landau 2011). Similar results were found in these two studies even though different methods were used to exclude insect predators (mesh cloth vs. insecticide). In *Luehea*, physical dormancy arises from an impermeable seed coat (Acuña & Garwood 1987, Sautu *et al.* 2007). Insect seed predators can break seed physical dormancy by probing or scarifying impermeable seed coat/seed covering parts without killing the seeds (Karban & Lowenberg 1992, Vallejo-Marín *et al.* 2006, Fox *et al.* 2012).

The fraction of initially dormant seeds in this study (on average 45%) did not differ between trees from PNM and Gamboa and was similar to that reported by Sautu *et al.* (2006). The adaptive significance of seed dormancy for tropical pioneers remains unclear. While seed persistence in the soil is adaptive in allowing seeds to recruit from the seed bank after gaps occur, dormancy may prevent seeds from germinating in favorable recruitment sites and would be disadvantageous unless it provides a protective role preventing pathogen transmission to seed tissue or reduces the ability of seed predators to locate seeds (Dalling *et al.* 1997b, 2011, Paulsen *et al.* 2013). In this study, we found an increase in the fraction of seeds that were dormant after incubation in the soil. Therefore, the finding suggests a protective role of seed dormancy for seed survival.

Effect of sham exclosures on capsule maturation.—We found different effects of sham mesh exclosures on the proportion of mature capsules across sites. The presence of cut nylon mesh positively affected the development of capsules in Gamboa but negatively affected capsule production in PNM. The presence of cut mesh may alter the microclimate conditions of the shoots preferentially for capsule development in Gamboa. Previous studies have shown that mesh bagging can alter the microclimate relative to unenclosed samples. Mesh cloth may reduce light intensity, temperature, and wind speed and may increase humidity inside the bags (Smith & De Bach 1942, Hand & Keaster 1967). However, some studies have reported that no differences are found in temperature and humidity between bagged and unbagged treatments (Hand & Keaster 1967, Nelson & Rieske 2014).

CONCLUSION

Here, we show how interactions of seeds with pathogens and predators in the forest canopy can affect seed dispersal, seed dormancy, and germination from the soil seed bank. These effects were context dependent, with more negative effects of fungal pathogens on seed development in the wetter site. Although our experimental treatments had significant effects on seed fate, variation in seed predation was highly variable in both *Luehea* populations at the individual branch and tree level. Sources of variation in seed losses at local scales in forest canopies, and their potential link to intrinsic plant traits, remain a largely unexplored area of the reproductive ecology of tropical trees.

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DATA AVAILABILITY

Data available in the Dryad Repository: http://dx.doi.org/10.5061/dryad.b12p5 (Tiansawat et al. 2017)

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article:

APPENDIX S1. Seed collection.

TABLE S1. *Number of experimental shoots applied to the given trees and number of seed collections in two sites.*

TABLE S2. *Summary of GLMM for the proportion of apparently healthy, mature capsules that developed in the canopy.*

TABLE S3. *Summary of GLMM for the proportion of germinated, fresh seeds collected from the canopy.*

TABLE S4. *Summary of GLMM for the proportion of dormant, fresh seeds collected from the canopy.*

TABLE S5. *Summary of GLMM for the proportion of germinated, buried seeds recovered from the soil.*

TABLE S6. *Summary of GLMM for the proportion of dormant, buried seeds recovered from the soil.*

FIGURE S1. The effect of natural enemy exclusion treatments on the probability of capsule maturation in *Luehea*.

FIGURE S2. The effect of natural enemy exclusion treatments on the probability that a canopy-collected *Luehea* seed germinates.

FIGURE S3. The effect of natural enemy exclusion treatments on the probability that a canopy-collected *Luehea* seed is dormant.

FIGURE S4. The effect of natural enemy exclusion treatments on the probability that a *Luehea* seed is dormant after burial.

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