Journal of Ecology

British Ecological Society

Journal of Ecology 2010, **98**, 147–155 doi: 10.1111/j.1365-2745.2009.01589.x

Interspecific variation in susceptibility to fungal pathogens in seeds of 10 tree species in the neotropical genus *Cecropia*

Rachel E. Gallery[1]*†, David J. P. Moore[2] and James W. Dalling[1,3,4]

[1]*Program in Ecology and Evolutionary Biology, University of Illinois, Urbana-Champaign, Urbana, IL 61801, USA;* [2]*Department of Geography, King's College London, Strand, London WC2R 2LS, UK;* [3]*Department of Plant Biology, University of Illinois, Urbana-Champaign, Urbana, IL 61801, USA; and* [4]*Smithsonian Tropical Research Institute, Apartado 0843-03092, Balboa, República de Panamá*

Summary

1. Species differences in susceptibility to pathogens acting at early life history stages may strongly influence the abundance and distribution of tropical trees. Here, we test the susceptibility of 10 congeners of the pioneer genus *Cecropia* to fungal seed and seedling pathogens and compare interspecific differences in intrinsic seed defences with survival.

2. Pathogens were experimentally removed through fungicide addition and/or autoclave sterilization of forest soil to determine the relative importance of fungal versus other microbial pathogens. Treatments were applied during a 4-month seed incubation (pre-emergence) phase or during an 8-week germination phase to distinguish between seed and seedling mortality.

3. Overall, seedling emergence after incubation in fungicide-treated, autoclaved soil was twice that in live soil, with significant positive effects of fungicide for six of 10 species. Pathogen infection occurred while seeds were quiescent in soil; fungicide addition during germination had no effect on emergence. Seedling emergence after burial ranged from 6 to 58%, indicating large interspecific variation in the capacity for *Cecropia* seeds to persist in the seed bank. Neither interspecific variation in survivorship, nor the relative strength of fungicide effects on survivorship was correlated with seed defence traits.

4. For four species, measurements of fungicide effects on emergence were coupled with direct measurements of the fungal and bacterial infection of seeds and seedlings. For two species, fungicide addition resulted in lower fungal infection rates and higher emergence success. However, *Cecropia peltata*, the species with the highest overall emergence success, also had the highest fungal infection rate. This suggests that either *C. peltata* was infected by a different suite of fungi than other congeners, or that fungi had low pathogenicity when colonizing this host species.

5. *Synthesis*. Our study shows strong interspecific variation in seed survival and susceptibility to fungal infection among congeneric tree species with similar life history. These differences are likely to influence recruitment success from the soil seed bank and may play a role in species coexistence.

Key-words: bacteria, Ecuador, fungi, Panama, plant defences, recruitment, seed bank, species coexistence, tannins, tropical forest

Introduction

Mortality at seed and seedling stages regulates adult tree abundance and distribution within communities (Wills *et al.* 1997; Hubbell *et al.* 1999; Harms *et al.* 2000; Connell *et al.* 2005; Wright *et al.* 2005). Seeds and seedlings are susceptible to a diverse array of predators, pathogens and herbivores whose host affinities, foraging patterns and exploitation of plant resources can have dramatically different outcomes on seedling distributions (e.g. Janzen 1970; Augspurger & Katajima 1992). An understanding of the contribution of different sources of juvenile mortality, including the potential for local biotic and abiotic factors to influence mortality rates, can therefore provide important insights into

*Correspondence author. E-mail: rachel.gallery@gmail.com
†Present address: Department of Zoology, University of Oxford, Oxford OX1 3PS, UK.

the variation in establishment potential of species in heterogeneous environments.

Fungal saprophytes and pathogens are ubiquitous in soils and have been identified as causal mortality agents for seed-banking species in most terrestrial ecosystems (Baskin & Baskin 1998; Agrios 2005). The magnitude of seed losses to fungal pathogens varies across species and systems: from 10 to 30% of observed seed mortality in temperate and subtropical grassland and forest communities (Lonsdale 1993; Masaki, Shibata & Nakashizuka 1998; Leishman *et al*. 2000; Blaney & Kotanen 2002) to nearly half of the observed seed mortality for certain pioneer species in temperate and neotropical forests (Dalling, Swaine & Garwood 1998; O'Hanlon-Manners & Kotanen 2004; Kotanen 2007; this study). Differences in the magnitude of these effects across systems may result from interspecific differences in susceptibility to pathogens, differences in pathogen communities and population densities, and environmental conditions (e.g. soil temperature and moisture) that may influence pathogen infection and spread.

Pathogenic fungi and Oomycota (fungus-like organisms in the Stramenopiles) are the predominant mortality agents of many tropical seeds and seedlings (Dalling, Swaine & Garwood 1998; Gilbert 2002; Hood, Swaine & Mason 2004; Gilbert 2005; Bell *et al*. 2006). Fungi, like other infectious agents, frequently demonstrate host specialization via differential infection rates or disease development among potential hosts (Burdon & Chilvers 1982; Kirchner & Roy 2000; Agrios 2005). The rapid growth rates and short generation times of fungal pathogens (Burdon & Chilvers 1982; Bruehl 1987) can lead to a rapid build-up of inoculum, facilitating infection and transmission among hosts (Maude 1996).

High probability of infection by pathogens is likely to select for anti-microbial traits in seeds and seedlings. Seeds contain various chemical and physical properties to defend against predators and pathogens (Janzen 1971, 1978; Hendry *et al*. 1994; Baskin & Baskin 1998; Veldman *et al*. 2007). Protective structures surrounding the seed may provide important physical barriers to fungal pathogen infection, while chemical constituents of the seed coat (e.g. seed wall, pericarp) such as alkaloids, flavonoids and phenolic acids may prevent or slow infection by inhibiting spore germination and hyphal growth (Fellows & Roeth 1992; Siemens, Johnson & Ribardo 1992; Kantar, Hebblethwaite & Pilbeam 1996). Symptom development from pathogen infection of mature seeds may depend upon variation in nutritional status during seed development or damage to the seed coat during dispersal, which could increase susceptibility to infection (Wulff 1986; Kremer & Spencer 1989; Fenner & Thompson 2005; Levey *et al*. 2007).

Alternatively, pathogen infection may occur once seeds begin to germinate and protective seed structures are ruptured (i.e. fatal germination, Fenner & Thompson 2005; see also Burdon & Shattock 1980; Neher *et al*. 1988). Under this scenario, germination speed and chemical defensive properties of the embryo and endosperm may be more important determinants of infection and survival. Previous studies have assumed that mortality in seed banks occurs when quiescent seeds become infected by pathogenic fungi (Dalling, Swaine & Garwood 1998; Gallery *et al*. 2007b; Gallery, Dalling & Arnold 2007a). However, assessments of seed losses in the seed bank are based on counts of emerging seedlings under favourable germination conditions and cannot distinguish between fatal infections of ungerminated and germinating seeds.

Seed loss to post-dispersal fungal infection is likely to be high for seeds that persist for prolonged periods before germination (Gilbert 2002). In mature tropical forests, seed persistence in seed banks for months to decades is common among pioneer species, which require canopy gap conditions to trigger germination (Vázquez-Yanes & Smith 1982; Pearson *et al*. 2002; Dalling & Brown 2009). Seeds of pioneer species that are dispersed to the forest understorey are therefore particularly susceptible to infection, as high soil moisture and temperature favour fungal growth, while low irradiance and red: far-red ratio of irradiance inhibit germination (Augspurger 1984; Augspurger & Kelly 1984; Vázquez-Yanes & Orozco-Segovia 1993; O'Hanlon-Manners & Kotanen 2004).

In this study, we compared the frequency and timing of fungus-induced mortality among a suite of species in the neotropical genus *Cecropia*. While *Cecropia* seeds are common in the seed bank, reports on the ability of seeds to persist in the soil seed bank vary dramatically (Holthuijzen & Boerboom 1982; Dalling, Swaine & Garwood 1997; Murray & Garcia 2002; Alvarez-Buylla & Martínez-Ramos 1990). For other taxa, properties of seeds that potentially protect against pathogen infection, such as seed coat thickness and presence of chemical defences, are correlated with persistence in the seed bank (Hendry *et al*. 1994; Murray & Garcia 2002; Orrock & Damschen 2005; Veldman *et al*. 2007). There is some evidence that *Cecropia* species vary in the concentration of tannins in the pericarp (Lobova *et al*. 2003), and in seed coat thickness (Pearson *et al*. 2002). However, effects of interspecific differences in tannin concentrations, seed mass or endocarp and pericarp thickness on susceptibility to pathogens and thereby on seed persistence in soil have not been examined in *Cecropia*.

Here, we examine the overall importance of pathogens and the relative importance of fungal pathogens in *Cecropia* emergence success. We compare pathogen infection at the pre-germination versus germination and emergence stage, and explore whether physical and chemical seed defences contribute to seedling emergence success. Finally, we discuss the potential for interspecific differences in tolerance to fungal infection for species coexistence in communities.

Materials and methods

STUDY SPECIES

Cecropia (Cecropiaceae) comprises 61 pioneer species restricted to the neotropics (Berg, Franco-Roselli & Davidson 2005). Our focal species (Table 1) are dioecious pioneer trees whose fruit are borne in a fleshy, catkin-like perianth and are dispersed by birds, bats and primates (Croat 1978; Milton 1991; Lobova *et al*. 2003).

In July and September 2005, seeds were collected from the canopy of six individuals of each of four *Cecropia* species growing in the Barro Colorado Nature Monument (BCNM) in central Panama (9°9′ N, 79°51′ W; see Leigh, Rand & Windsor 1996) and six *Cecropia* species

Table 1. Fixed-effect ANOVA results for seedling emergence. Factors include fungicide (FF, FW, WF and WW), soil (live, sterile), species effects and their interactions

Factor	d.f.	*F*-value	Pr > F
Fungicide	3	4.41	0.0047
Soil	1	29.54	< .0001
Species	9	18.36	< .0001
Species × fungicide	27	0.78	0.7775
Soil × fungicide	1	4.4	0.0368
Species × soil	9	0.84	0.5767
Species × soil × fungicide	9	0.75	0.6667
Error	300		

from the Yasuni National Park in the Ecuadorian Amazon (1°26′ S, 75°40′ W, see Valencia *et al*. 2004). Immediately after collection, seeds were removed from infructescences and rinsed in a 10% Clorox (0.5% sodium hypochlorite) solution for 1 min to remove pulp and surface contaminants. Seeds were then air-dried under sterile conditions under low red : far red irradiance and seeds from the six individuals per species were pooled.

FUNGICIDE EXPERIMENT

We used a fungicide addition experiment, with BCNM forest soil as an inoculum source, to test the effects of soil-borne pathogens and predators on seed and seedling survival of the 10 *Cecropia* species. The experiment was divided into two stages: (i) the seed incubation (pre-emergence) phase and (ii) the germination phase. Fungicide was applied either during seed incubation or during seedling emergence, thus distinguishing between fungus-induced seed and seedling mortality.

Fungicide addition during seed incubation

At the onset of the experiment in September 2005, the top 10 cm of soil was collected from 0.5-m^2 plots at six randomly chosen sites on Barro Colorado Island, BCNM. Sites were at least 50 m from any *Cecropia* tree. Soil samples were pooled, thoroughly mixed and divided into half; half of the soil was sterilized (autoclaved at 115 °C for 2 h). Two hundred and forty sterile 250-mL pots were filled with untreated, live field soil. Nylon mesh seed bags (0.5-mm mesh size) were filled with 30 seeds of a single species mixed with 10 g of autoclave-sterilized soil, and one seed bag was buried at 3 cm depth in each pot. Pots were randomly assigned either water or fungicide addition. For each species, we used a total of 24 seed bags (10 species × 24 seed bags = 240 pots). Half of the bags received fungicide during the seed incubation phase, while the remaining 12 bags received a water control. During the germination phase, half of the original fungicide (F) and water (W) bags received fungicide addition (FF, WF) and half received water (FW, WW). Thus, six seed bags per species received each treatment.

An autoclave-sterilized soil treatment was included to compare the relative importance of fungal versus other microbial pathogens and seed predators. Additionally, autoclave sterilization with and without fungicide addition was used to test for potentially negative effects of fungicide addition on seedling survival. One hundred and twenty sterile 250-mL pots were filled with autoclave-sterilized soil (at 115 °C for 2 h). Autoclaving kills macro- and micro-invertebrates, and most fungal and bacterial propagules in soil (Kendrick 2000). For fungicide additions, seed bags in the autoclaved soil treatment received either the FF or WW additions (n = 6 bags per species).

We conducted a separate pilot study with the four *Cecropia* species from Panama to ensure that Captan fungicide (Orthocide 50%, Wettable Powder; Micro Flo LLC, Memphis, TN, USA) was not toxic to seeds or seedlings in the concentration applied in the main experiment. A 1 : 100 solution of Captan in sterile water was applied to two sterile, paper-lined Petri dishes containing seeds of each of the four species (n = 3 plates per species). Sterile water was added to similar Petri dishes for each species (n = 3 plates per species) as a control. Petri dishes each contained 30 seeds and were stored in ambient laboratory conditions (26 °C, 12 h light–dark cycle). Germination was measured weekly, for 8 weeks, and the proportion of live seedlings at the end of 8 weeks (of 30 original seeds) was averaged by species (n = 2). Captan had no observable effect on germination and survival compared with the control (Student's paired two-tailed t-test; $P > 0.7$ for every species).

Pots were stored in the dark in an ambient-air shade house (mean maximum = 30.8 ± 1.0 °C, mean minimum = 23.9 ± 0.8 °C) for 120 days. For the initial fungicide application, 25 mL of a 1 : 100 solution of Captan in water was applied to each pot (10 g Captan L^{-1} water). Subsequently, water (25 mL) or fungicide (25 mL of 5 g Captan L^{-1}) was applied every 10 days. Captan (*N*-trichloromethylthio-4-cyclohexene-1, 2 dicarboximide) is a non-systemic heterocyclic fungicide effective against many seed-rotting Ascomycota, Basidiomycota and Oomycota (Neergaard 1977) that has been shown to significantly reduce seed mortality in tropical and temperate soils (Dalling, Swaine & Garwood 1998; Blaney & Kotanen 2001, 2002; Schafer & Kotanen 2003; O'Hanlon-Manners & Kotanen 2004; Orrock & Damschen 2005).

Fungicide addition during germination trials

After 120 days of incubation, the seed bags were retrieved and the soil in which they had been buried was discarded. We collected fresh field soil in January 2006 and filled 240 pots with live soil and 120 pots with autoclaved soil as before. Seed bags were cut open and secured onto the top layer of soil in the pots to serve as a barrier between the bag contents and field soil, thus ensuring only the experimental seeds were counted during the germination trials.

Pots were returned to an ambient-air shade house with 30% full sunlight with high red : far red irradiance (> 0.9) to induce germination (Vázquez-Yanes & Smith 1982; Pearson *et al*. 2002). Half of the original fungicide (F) and water (W) bags received fungicide addition (FF, WF) and half received water (FW, WW; n = 6 bags per species per treatment). Water or fungicide was applied, and germination was measured, every 7 days over an 8-week period, which is sufficient for *Cecropia* emergence (2–6 weeks; Dalling, Swaine & Garwood 1995). Seedling emergence was measured as the proportion of 30 seeds per pot that produced seedlings with symptom-free, fully expanded cotyledons adjusted for the initial seed viability which was determined at the onset of the experiment with a subsample of 100 seeds per species ([Final/Initial], Table 1). *Cecropia marginalis*, the smallest-seeded species in this study, had very low initial viability (12%), indicating poor seed lot quality. *Cecropia engleriana*, one of the larger-seeded species, also had low initial viability (38%), whereas initial viability of the remaining species was ≥50%.

MEASURING SEED TRAITS AND INFECTION

Seed mass and seed coat thickness measurements followed Pearson *et al*. (2002) (Table 1). Protocol followed Porter, Hrstich & Chan

(1986) for the proanthocyanidine test measuring condensed tannins (CT). Protocol followed Hagerman (1987) for the radial diffusion assay, which is a protein-binding assay that measures the biological activity of both condensed and hydrolysable tannins (HT). Results are reported as a percentage of quebracho units (QU), which was the standard used for both assays. For each species, we used the mean of two replicate samples for statistical analysis.

We tested the efficacy of fungicide addition in reducing fungal infection by comparing the proportion of seeds and seedlings that yielded a fungal isolate in culture under the different fungicide treatments. Overall, survival was highest in the autoclave-sterilized soil treatment, suggesting that *Cecropia* seed and seedling pathogens were killed during sterilization. Thus, we examined infection of seeds and seedlings in live soil only. We focused on the four *Cecropia* species from Panama, which represented a range in seedling emergence, and at the end of the 8-week germination period a subsample of four randomly selected pots per species per fungicide treatment (FF, WW) were chosen. Three seedlings (where available) were removed from each pot, and the remaining soil was wet-sieved to collect three ungerminated seeds (where available) from the same pot. The roots were cut from the seedlings, and roots and seeds were thoroughly rinsed in tap water to remove soil. Both seeds and roots were surfaced-sterilized by agitation in 95% ethanol (15 s), followed by 10% Clorox (0.5% sodium hypochlorite; 2 min) and then 70% ethanol (2 min; protocol follows Arnold *et al.* 2000). Using sterile techniques, seeds were plated intact and roots were cut into small pieces and plated into 1.5-mL vial slants with 2% malt extract agar (MEA), which is a general medium that encourages growth by diverse microfungi, although it likely underestimates the number of fungi present (e.g. Arnold & Lutzoni 2007). Therefore, we use the term 'infection' to represent the number of colonies isolated from seed and root tissue, although this is a conservative estimate of actual infection. Previous results showed no difference in the frequency of fungal growth from *Cecropia* seeds when plated intact versus cut in half (A. E. Arnold, personal communication). A total of 70 seedling roots (n = 24 *Cecropia longipes* and *Cecropia peltata* roots each, 16 *Cecropia obtusifolia* roots and six *Cecropia insignis* roots) and 88 seeds (n = 24 *C. insignis* and *C. longipes* each, 23 *C. obtusifolia* seeds and 17 *C. peltata* seeds) were individually plated; vials were sealed with Parafilm and stored at 26 °C in a 12 h light–dark cycle. Evidence of culture growth was monitored under a stereoscope over 7 days.

When evidence of microbial growth was observed, temporary slide mounts were used to determine whether the culture had produced fungal spores and whether bacteria were present. At the end of the observation period, vials contained either sterile fungal mycelia, bacterial rods, both bacteria and fungal mycelia, or no culture growth. Taxonomic identifications were not attempted.

DATA ANALYSES

A fixed-effect ANOVA with type III sums of squares (PROC MIXED; SAS Institute v. 9.1, Cary, NC, USA) was used to test fungicide treatments (FF, FW, WF and WW), autoclave-sterilization (live, sterile), species effects and their interactions on seedling emergence. Planned comparisons of the timing of fungicide addition were analysed with Tukey–Kramer-adjusted (α = 0.05) linear contrasts. Wilcoxon–Mann–Whitney U-tests were used to compare infection rates of seeds and roots since these samples could not be considered independent. We determined the relationship among seed traits, pathogen susceptibility and pathogen tolerance using Spearman's rank correlations.

Results

PATHOGEN REMOVAL INCREASES SEEDLING EMERGENCE

Soil sterilization combined with fungicide addition nearly doubled seedling emergence from 31.2 ± 3.1% [(mean ± SE) live & WW] to 59.1 ± 4.0% (sterile and FF; $t_{1,300}$ = 6.11, P < 0.0001). When the treatments were applied separately, emergence was not significantly different between the autoclave-sterilized and fungicide-treated soil (sterile and WW versus live & FF: $t_{1,300}$ = 1.57, P = 0.62). Seedling emergence in untreated soil (live and WW soil) varied from 5.8 ± 2.7% (*C. obtusifolia*) to 57.2 ± 8.8% (*C. peltata*). This 10-fold difference in seedling emergence (Fig. 1) despite similar initial seed viability in these two species indicates large interspecific variation in the capacity for *Cecropia* seeds to persist in the seed bank.

In this study, fungi were the predominant source of mortality for *Cecropia*. Fungicide addition significantly improved seedling emergence overall (live and FF versus live and WW: $t_{1,300}$ = 3.75, P < 0.01). There was no interaction between species and soil treatment (Table 1); mean emergence was always greater in the absence of pathogens (sterile and FF treatment) and this was significant in six of the 10 species (Fig. 1). There was no evidence that species with low emergence success in the presence of pathogens benefited more from fungicide addition than those with high emergence success in the presence of pathogens (Fig. 2: slope not different from 1 : 1 regression for the different combinations of soil sterilization and fungicide addition; sterile/FF $t_{1,8}$ = 0.37, P = 0.72).

To test whether *Cecropia* species are more susceptible to fungal pathogens at the pre-emergence or germination and emergence stage, we varied the timing of fungicide application

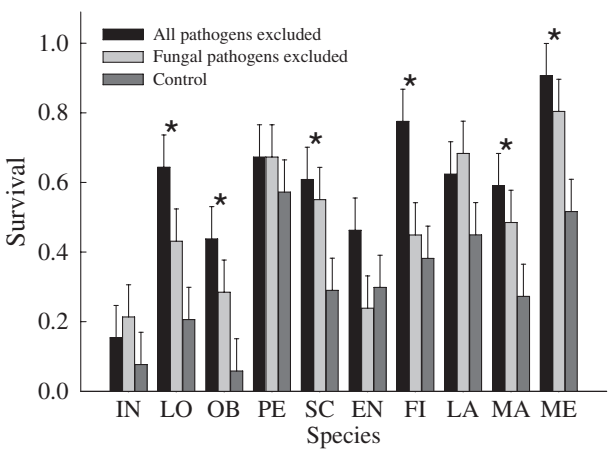

Fig. 1. Differences in the proportion of seedling emergence for 10 *Cecropia* species when all pathogens are excluded (black bars), only fungal pathogens are excluded (light grey bars) or no pathogens are excluded (control; dark grey bars). On the x-axis, species names are coded by the first two letters and the four species from Panama (IN, LO, OB, PE) are presented first. *Significant differences between pathogen exclusion and controls. Bars represent lease squares mean; error bars represent + 1 SE of the mean.

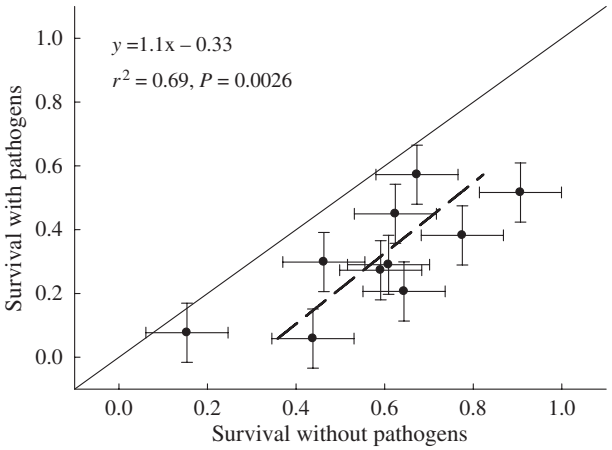

Fig. 2. Geometric mean regressions of seedling emergence in the presence and absence of pathogens (Model II; using MATLAB 7.1 (*r*) for Windows (MathWorks Inc., Natick, MA, USA). Data points represent species mean.

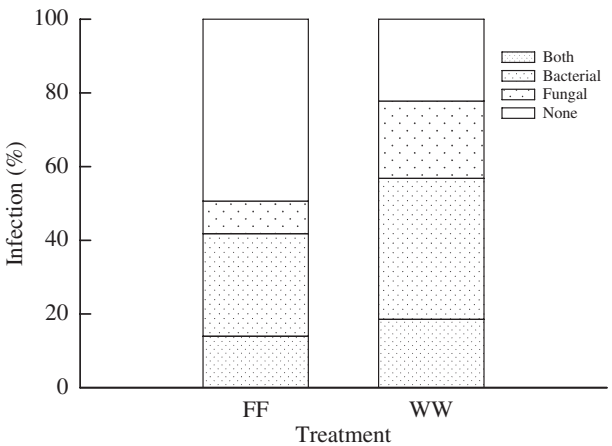

Fig. 4. Percentage of roots and seeds of four *Cecropia* species that yielded fungal and/or bacterial isolates after fungicide (FF; $n = 80$) or water (WW; $n = 78$) addition to seed bags buried in live soil.

to live soil. We found a significant positive effect of applying fungicide to seeds (Fig. 3: live WW versus live FW, $t_{1,200} = 3.04$, $P < 0.01$), but not of applying fungicide only during seedling emergence (Fig. 3: live WW versus live WF, $t_{1,200} = 1.31$, $P = 0.19$).

VARIATION IN INFECTION TOLERANCE AMONG SPECIES

For the four BCNM species, which includes those with the highest and lowest overall emergence, we examined the effect of fungicide addition on microbial infection rates. Overall, Captan fungicide addition significantly lowered both fungal and bacterial infection of seeds and roots relative to the untreated controls (Fig. 4). After 7 days of incubation on 2% MEA, 50% ($n = 80$) of seeds and roots from the fungicide addition treatment (FF) yielded microbial growth in culture, while microbial growth was observed in 88% ($n = 78$) of seeds and roots in the untreated soils (WW). Samples were pooled from both life stages (seed and root) of all four species to test the effect of fungicide addition on lowering overall infection rates; there was no difference in incidence of infection of seeds and roots (FF: $\chi^2 = 0.097$, d.f. = 1, $P = 0.75$; WW: $\chi^2 = 2.07$, d.f. = 1, $P = 0.15$). Fungal infection was significantly lower in fungicide-treated soils (Wilcoxon–Mann–Whitney *U*-test, $z = -2.16$, $P < 0.05$); fungi accounted for 23% of the isolates from the fungicide addition and 40% of isolates from untreated soils. Bacterial infection was significantly higher than fungal infection in both soil treatments (Fig. 4). However, fungicide addition also lowered bacterial infection relative to untreated soils (42% and 58%, respectively; $z = -1.81$, $P < 0.05$).

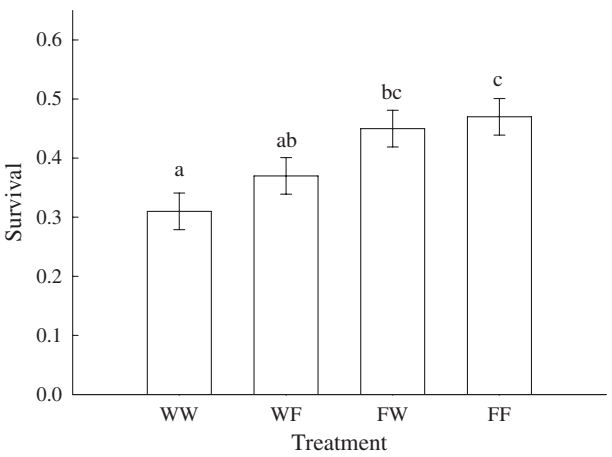

Fig. 3. Proportion of seedlings emerging under different fungicide (FF, FW, WF and WW) treatments. The F and W variables on the *x*-axis correspond to the fungicide treatments during the seed incubation (first variable) and germination (second variable) phases of the experiment. For example, FF indicates that fungicide was applied at both phases of the experiment, while FW indicates that fungicide was only applied during the seed incubation phase. Bars represent least squares mean; error bars represent + 1 SE of the mean.

Differences in seedling emergence among the four BCNM *Cecropia* species may be partially explained by differences in susceptibility to, or tolerance of, fungal pathogens. Interspecific variation in both infection levels and seedling emergence was high. Fungicide addition significantly increased *C. obtusifolia* survival (live FF versus live WW; $t_{1,40} = 7.41$, $P < 0.01$) and reduced fungal infection relative to the untreated control ($n = 28$, two-tailed Fisher's exact test: $P < 0.01$; Fig. 5). *Cecropia obtusifolia* had the lowest emergence of all species in live soil (6%), and 44% of roots and seeds yielded a fungal isolate. Similarly, *C. insignis* had significantly higher emergence (live FF versus live WW; $t_{1,40} = 2.71$, $P < 0.05$) and lower fungal infection with fungicide addition ($n = 15$, two-tailed Fisher's exact test: $P < 0.001$; Fig. 5).

In contrast, fungal infection did not have a strong negative effect on seedling emergence for *C. longipes* and *C. peltata*. While *C. longipes* had higher emergence with fungicide addition (live FF versus live WW; $t_{1,40} = 7.33$, $P < 0.01$), fungal infection levels were not significantly different between the two treatments ($n = 29$, two-tailed Fisher's exact test: $P = 0.16$).

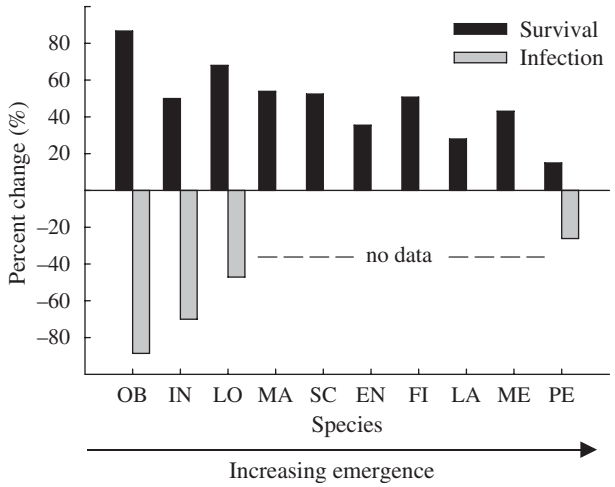

Fig. 5. Effect of fungicide addition on per cent increase in survival (positive black bars) and per cent decrease in fungal infection (negative grey bars; measured in four species only). Species are listed from lowest to highest overall emergence in live, untreated soil.

The species with the highest emergence in live soils, *C. peltata* (58%), did not experience higher emergence with fungicide addition (live FF versus live WW; $t_{1,40} = 1.47$, $P = 0.12$). *Cecropia peltata* seed and seedlings also had the highest infection rates of all species in live soils (65%), and fungicide addition did not significantly reduce infection ($n = 31$, two-tailed Fisher's exact test: $P = 0.14$; Fig. 5).

SEED CHARACTERISTICS AND SEEDLING EMERGENCE

Species varied in CT and HT activity. For six species, CT values were $< 2\%$, values considered too dilute to serve as important lines of defence against fungi or other microbes and invertebrates (Makkar 2003; Table 2). Radial diffusion analyses showed the biological activity of CT and/or HT to be highest in *C. peltata, C. obtusifolia* and *C. membranaceae* (Table 2). These are likely to be the only species for which the biological activity of tannins is strong enough to affect fungi (N. Conklin, personal communication).

To test the hypothesis that differences in seed characteristics influence pathogen-mediated survival, we compared seed mass, seed coat thickness and investment in tannins with pathogen susceptibility and seedling emergence. Seed coat thickness was positively correlated with seed mass (Spearman's rank correlation, $r_s = 0.87$, $n = 10$, $P < 0.001$; Table 2). There was no relationship between investment in tannins and seed mass or seed coat thickness ($r_s = -0.13$, $n = 10$, $P = 0.72$; Table 2). To measure the effectiveness of investment in physical and chemical defences against pathogen-caused mortality, we compared the magnitude of the fungicide effect on seedling emergence (FF–WW) with tannin activity and seed coat thickness. Neither seed coat thickness ($r_s = -0.24$, $n = 10$, $P = 0.50$) nor investment in tannins ($r_s = 0.02$, $n = 10$, $P = 0.95$) was correlated with susceptibility to pathogens.

Discussion

STRENGTH AND TIMING OF SEED MORTALITY ATTRIBUTABLE TO FUNGAL PATHOGENS

In this study, fungal pathogens were the predominant source of mortality for *Cecropia*. For six species, there was no difference in survival when all pathogens were removed through autoclave sterilization combined with fungicide addition versus when only fungal pathogens were removed (Fig. 1). Overall, survival was highest when all potential pathogens were removed, suggesting other microbial pathogens are present in this system as well.

In ecological studies of seed banks, distinguishing between pre- and post-emergence mortality provides insight into selection pressures operating at different life history stages. In this study, fungicide addition lowered fungal infection of both seeds and seedlings; however, a significant positive fungicide effect on seedling emergence only occurred when fungicide was applied to seeds. The duration of the seedling emergence stage

Table 2. Seed mass, pericarp thickness, condensed tannin (CT), radial diffusion of hydrolysable tannins (RD) and per cent initial viability of 10 *Cecropia* species from Barro Colorado Nature Monument, Panama, and Yasuni National Park, Ecuador

	Provenance	Seed mass (mg)*	Seed coat thickness (μm)†	CT (% QU)	RD (% QU)	Initial viability (%)
Cecropia engleriana (Snethl.)	Ecuador	1.19 ± 0.15	119 ± 3.9	0.49	0.00	37.2
Cecropia ficifolia (Warb. ex Snethl.)	Ecuador	0.58 ± 0.06	87 ± 1.5	2.35	3.24	49.4
Cecropia insignis (Lieb.)	Panama	0.57 ± 0.07	85 ± 4.1	0.15	0.00	65.0
Cecropia latiloba (Miq.)	Ecuador	1.33 ± 0.23	104 ± 1.2	1.39	4.62	82.8
Cecropia longipes (Pitt.)	Panama	1.09 ± 0.12	92 ± 4.7	0.40	0.00	88.9
Cecropia marginalis (Cuatr.)	Ecuador	0.34 ± 0.05	70 ± 2.5	0.60	0.00	12.2
Cecropia membranaceae (Trécul)	Ecuador	0.68 ± 0.07	85 ± 3.2	8.70‡	8.79‡	50.6
Cecropia obtusifolia (Bertol.)	Panama	0.52 ± 0.03	71 ± 2.3	6.40‡	9.58‡	76.1
Cecropia peltata (L.)	Panama	0.79 ± 0.11	147 ± 4.6	9.38‡	12.95‡	88.3
Cecropia sciadophylla (Mart.)	Ecuador	1.61 ± 0.10	195 ± 4.7	0.27	0.00	76.7

*Mean ($n = 20$) ± 1 SE.
†Mean ($n = 10$) ± 1 SE.
‡Biologically active levels in % quebracho units (QU) standard.

is short (2–4 weeks) compared with seed incubation period (4 months in this experiment), which would provide less opportunity for pathogens to encounter and invade vulnerable seedling tissue. Alternatively, chemical defences may be induced during germination, thus providing greater protection to seedlings. While fungicides can remove beneficial root-colonizing fungi [such as arbuscular mycorrhizas (AM)], our measure of successful germination in this study was cotyledon expansion and we do not expect strong AM fungi effects at this stage. While certain fungi are capable of infecting both seed and seedling tissue in *Cecropia* (U'Ren *et al*. 2009) and other species (Masaki & Nakashizuka 2002; Agrios 2005), these results suggest that pre-emergence pathogen infection of *Cecropia* seeds is more important than infection of emerging seedlings.

INTERSPECIFIC VARIATION IN SUSCEPTIBILITY TO FUNGAL PATHOGENS AND TOLERANCE OF FUNGAL INFECTION

In this study, the 10 *Cecropia* species had significantly different seedling emergence success when incubated in the same forest soil and, consequently, with a similar inoculum source. These interspecific differences in the capacity of seeds to survive in the soil may arise from many factors including: (i) differences in seed traits that confer defence, (ii) differences in susceptibility to infection to a common soil microbial community and (iii) differences in tolerance to fungal infection. Although the range in seed characteristics (seed mass, pericarp thickness and tannin activity) is relatively small among the *Cecropia* species included in this study (Table 2), species show large differences in persistence in soil and susceptibility to fungal infection (Figs 1 and 5).

Differences in seed persistence may result from differences in seed defence traits such as resistance to microbial degradation of the pericarp during soil incubation. Defence characteristics may be physical, with seed coats lowering porosity and seed 'leakiness' and/or providing a physical barrier to hyphal growth. Anti-microbial compounds in the seed coat can also prevent infection. Compared with other tropical seed-banking tree species, *Cecropia* species do not appear to invest heavily in seed defences. Although we did not look at the full suite of potentially anti-fungal chemical seed defences, which may include chitinases, alkaloids and flavonoids (Scalbert 1991; Welbaum 2006), we found interspecific differences in the thickness of protective structures surrounding the seed and in tannin activity among *Cecropia* species (Table 1; see also Lobova *et al*. 2003; Pearson *et al*. 2002).

In this study, species provenance (Panama, Ecuador) may have played a stronger role than physical or chemical defences in influencing species-specific differences in pathogen-mediated seed mortality. Pathogen removal significantly increased survival in seeds from both Panama and Ecuador (Fig. 1). However, the majority of plants are not susceptible to most plant pathogens (Agrios 2005; see also review in Parker & Gilbert 2004) suggesting that seeds from Ecuador may have been less susceptible to novel pathogens from Panama soil. The Ecuador seeds represented the largest range in seed traits, which could partially explain the lack of relationship between seed characteristics and pathogen susceptibility.

Measuring microbial infection provided the opportunity to explore whether differences in the magnitude of fungicide effects were caused by differences in microbial colonization or differences in susceptibility to lethal infection. Fungicide addition significantly lowered both fungal and bacterial infection of seeds and roots. It is unclear whether bacteria are contaminants that colonized seeds after incubation in the soil, or why fungicide addition would lower bacterial colonization of seeds and seedlings. For *C. obtusifolia* and *C. insignis*, the negative correlation between seedling emergence and fungal infection suggests some of the seed- and root-infecting fungi in this study were pathogenic. In contrast, fungal infection did not have a strong negative effect on *C. longipes* or *C. peltata* seedling emergence. High survival coupled with high fungal infection rates suggests that *C. longipes* and *C. peltata* are tolerant of fungal infection that some of the fungi infecting these species were not pathogenic and that seed survival may also be mediated by mutualistic fungal infection.

Species differences in survival may result from host affinities of pathogens or differences in susceptibility to a common soil microbial community. In a common garden study, the four fungal genotypes most commonly isolated from buried seeds of four *Cecropia* species accounted for almost 50% of all genotypes encountered and were recovered from all *Cecropia* species (Gallery, Dalling & Arnold 2007a; Gallery *et al*. 2007b). At the same time, other commonly isolated fungi demonstrated strong host affinities, indicating that host-specific interactions among fungi and seeds are also common in the seed bank. The similarly high infection levels among the four *Cecropia* species, which were the same species used in this study, (80–95%; Gallery *et al*. 2007b; Gallery, Dalling & Arnold 2007a) indicate interspecific differences in species tolerance to infection or different pathogenicities of the infecting fungi. The fine-scale spatial structure of fungal communities in soils suggests that a seed is likely to encounter both host-specific and generalist fungi. Generalist fungi eliciting differential species responses (e.g. Schafer & Kotanen 2004; Augspurger & Wilkinson 2007; Kluger *et al*. 2008) could result in similar outcomes for species distributions as those generally predicted from species-specific pathogen interactions.

Acknowledgements

This research was supported by NSF DEB-0343953 to J.W.D and NSF Doctoral Dissertation Improvement Grant to R.E.G. We thank the Smithsonian Tropical Research Institute, Panama, and the Ministerio del Ambiente, Ecuador, for providing facilities, logistical support and permission to conduct this research. We thank Nancy Lou Conklin-Brittain and Meg Crowfoot, Harvard University, for assistance with tannin extractions and interpretations of results, and Evelyn Sanchez for field assistance. Finally, we wish to thank two anonymous referees for valuable comments.

References

Agrios, G.N. (2005) *Plant Pathology*, 5th edn. Elsevier Academic Press, San Diego, CA, USA.

Alvarez-Buylla, E. & Martínez-Ramos, R. (1990) Seed bank versus seed rain in the regeneration of a tropical pioneer tree. *Oecologia*, **84**, 314–325.

Arnold, A.E. & Lutzoni, F. (2007) Diversity and host range of foliar fungal endophytes: Are tropical leaves biodiversity hotspots? *Ecology*, **88**, 541–549.

Arnold, A.E., Maynard, Z., Gilbert, G.S., Coley, P.D. & Kursar, T.A. (2000) Are tropical fungal endophytes hyperdiverse? *Ecology Letters*, **3**, 267–274.

Augspurger, C.K. (1984) Seedling survival of tropical tree species: interactions of dispersal distance, light-gaps, and pathogens. *Ecology*, **65**, 1705–1712.

Augspurger, C.K. & Katajima, K. (1992) Experimental studies of seedling recruitment from contrasting seed distributions. *Ecology*, **73**, 1270–1284.

Augspurger, C.K. & Kelly, C.K. (1984) Pathogen mortality of tropical seedlings: experimental studies of the effects of dispersal distance, seedling density, and light conditions. *Oecologia*, **61**, 211–217.

Augspurger, C.K. & Wilkinson, H.T. (2007) Host specificity of pathogenic *Pythium* species: implications for tree species diversity. *Biotropica*, **39**, 702–708.

Baskin, C.C. & Baskin, J.M. (1998) *Seeds: Ecology, Biogeography, and Evolution of Dormancy and Germination*. Academic Press, San Diego, CA, USA.

Bell, T., Freckleton, R.P. & Lewis, O.T. (2006) Plant pathogens drive density-dependent seedling mortality in a tropical tree. *Ecology Letters*, **9**, 569–574.

Berg, C.C., Franco-Roselli, P. & Davidson, D.W. (2005) *Cecropia*. Flora Neotropica Monograph **94**. New York Botanical Garden Press, New York, NY, USA.

Blaney, C.S. & Kotanen, P.M. (2001) Effects of fungal pathogens on seeds of mature and exotic plants: a test using congeneric pairs. *Journal of Applied Ecology*, **38**, 1104–1113.

Blaney, C.S. & Kotanen, P.M. (2002) Persistence in the seed bank: an experimental comparison of native and alien plants. *Écoscience*, **9**, 509–517.

Bruehl, G.W. (1987) *Soilborne Plant Pathogens*. Macmillan Press, New York, NY, USA.

Burdon, J.J. & Chilvers, G.A. (1982) Host density as a factor in plant disease ecology. *Annual Review of Phytopathology*, **20**, 143–166.

Burdon, J.J. & Shattock, R.C. (1980) Disease in plant communities. *Applied Biology*, **5**, 145–219.

Connell, J.H., Debski, I., Gehring, C.A., Goldwasser, L., Green, P.T., Harms, K.E., Juniper, P. & Theimer, T. (2005) Dynamics of seedling recruitment in an Australian tropical rainforest. *Tropical Rainforests: Past, Present, and Future* (eds E. Bermingham, C.W. Dick & C. Moritz), pp. 486–506. Chicago University Press, Chicago, IL, USA.

Croat, T.B. (1978) *Flora of Barro Colorado Island*. Stanford University Press, Stanford, CA, USA.

Dalling, J.W. & Brown, T.A. (2009) Long-term persistence of pioneer seeds in tropical rain forest soil seed banks. *American Naturalist*, **173**, 531–535.

Dalling, J.W., Swaine, M.D. & Garwood, N.C. (1995) Effect of soil depth on seedling emergence in tropical soil seed bank investigations. *Functional Ecology*, **9**, 119–121.

Dalling, J.W., Swaine, M.D. & Garwood, N.C. (1997) Soil seed bank community dynamics in seasonally moist lowland tropical forest, Panama. *Journal of Tropical Ecology*, **13**, 659–680.

Dalling, J.W., Swaine, M.D. & Garwood, N.C. (1998) Dispersal patterns and soil seed bank dynamics of pioneer tree species in moist tropical forest, Panama. *Ecology*, **79**, 564–578.

Fellows, G.W. & Roeth, F.W. (1992) Factors influencing shattercane (*Sorghum bicolor*) seed survival. *Weed Science*, **40**, 434–440.

Fenner, M. & Thompson, K. (2005) *The Ecology of Seeds*. Cambridge University Press, Cambridge, UK.

Gallery, R.E., Dalling, J.W. & Arnold, A.E. (2007a) Diversity and demographic impact of seed-infecting fungi: a case study with neotropical *Cecropia* spp. *Ecology*, **88**, 582–588.

Gallery, R.E., Dalling, J.W., Wolfe, B.T. & Arnold, A.E. (2007b) The influence of seed source, habitat, and fungi on *Cecropia* seed survival in two neotropical forests. *Seed Dispersal: Theory and Its Application in a Changing World* (eds A.J. Dennis, R. Green, E.W. Schupp & D.A. Westcott), pp. 479–495. CAB International Press, Wallingford, UK.

Gilbert, G.S. (2002) Evolutionary ecology of plant diseases in natural ecosystems. *Annual Review of Phytopathology*, **40**, 13–43.

Gilbert, G.S. (2005) The dimensions of plant disease in tropical forests. *Biotic Interactions in the Tropics* (eds D.R.F.P. Burslem, M.A. Pinard & S. Hartley), pp. 141–164. Cambridge University Press, Cambridge, UK.

Hagerman, A.E. (1987) Radial diffusion method for determining tannin in plant extracts. *Journal of Chemical Ecology*, **13**, 437–449.

Harms, K.E., Wright, S.J., Calderón, O., Hernandez, A. & Herre, E.A. (2000) Pervasive density-dependent recruitment enhances seedling diversity in a tropical forest. *Nature*, **404**, 493–495.

Hendry, G.A., Thompson, F.K., Moss, C.J., Edwards, E. & Thorpe, P.C. (1994) Seed persistence – a correlation between seed longevity in the soil and ortho375 dihydroxyphenol concentration. *Functional Ecology*, **8**, 658–664.

Holthuijzen, A.M.A. & Boerboom, J.H.A. (1982) The *Cecropia* seedbank in the Surinam lowland rain forest. *Biotropica*, **14**, 62–68.

Hood, L.A., Swaine, M.D. & Mason, P.A. (2004) The influence of spatial patterns of damping-off disease and arbuscular mycorrhizal colonization on tree seedling establishment in Ghanaian tropical forest soil. *Journal of Ecology*, **92**, 816–823.

Hubbell, S.P., Foster, R.B., O'Brien, S.T., Harms, K.E., Condit, R., Wechsler, B., Wright, S.J. & Loo de Lao, S. (1999) Light gap disturbances, recruitment limitation, and tree diversity in a neotropical forest. *Science*, **283**, 554–557.

Janzen, D.H. (1970) Herbivores and the number of tree species in tropical forests. *American Naturalist*, **104**, 501–528.

Janzen, D.H. (1971) Seed Predation by Animals. *Annual Review of Ecology and Systematics*, **2**, 465–492.

Janzen, D.H. (1978) The ecology and evolutionary biology of seed chemistry as related to seed predation. *Biochemical Aspects of Plant and Animal Coevolution* (ed. J.B. Harborne), pp. 162–206. Academic Press, NewYork, NY, USA.

Kantar, F., Hebblethwaite, P.D. & Pilbeam, C.J. (1996) Factors influencing disease resistance in high and low tannin *Vicia fabia*. *Journal of Agricultural Science*, **127**, 83–88.

Kendrick, B. (2000) *The Fifth Kingdom*, 3rd edn. Focus Publishing, R. Pullins Company, Newburyport, MA, USA.

Kirchner, J.W. & Roy, B.A. (2000) Evolutionary implications of host-pathogen specificity: The fitness consequences of host life history traits. *Evolutionary Ecology*, **14**, 665–692.

Kluger, C.G., Dalling, J.W., Gallery, R.E., Sanchez, E., Weeks-Galindo, C. & Arnold, A.E. (2008) Prevalent host-generalism among fungi associated with seeds of four neotropical pioneer species. *Journal of Tropical Ecology*, **24**, 351–354.

Kotanen, P.M. (2007) Effects of fungal seed pathogens under conspecific and heterospecific trees in a temperate forest. *Canadian Journal of Botany*, **85**, 918–925.

Kremer, R.J. & Spencer, N.R. (1989) Interaction of insects, fungi and burial on velvetleaf (*Abutilon theophrasti*) seed viability. *Weed Technology*, **3**, 322–328.

Leigh, E.G. Jr, Rand, A.S. & Windsor, D.M. (1996) *The Ecology of a Tropical Forest*. Smithsonian Institution Press, Washington, DC, USA.

Leishman, M.R., Masters, G.J., Clarke, I.P. & Brown, V.K. (2000) Seed bank dynamics: the role of fungal pathogens and climate change. *Functional Ecology*, **14**, 293–299.

Levey, D.J., Tewksbury, J.J., Izhaki, I., Tsahar, E. & Haak, D.C. (2007) Evolutionary ecology of secondary compounds in ripe fruit: case studies with capsaicin and emodin. *Seed Dispersal: Theory and its Application in a Changing World* (eds A.J. Dennis, R. Green, E.W. Schupp & D.A. Westcott), pp. 37–58. CAB International Press, Wallingford, UK.

Lobova, T.A., Mori, S.A., Blanchard, F., Peckham, H. & Charles-Dominique, P. (2003) *Cecropia* as a food resource for bats in French Guiana and the significance of fruit structure in seed dispersal and longevity. *American Journal of Botany*, **90**, 388–403.

Lonsdale, W.M. (1993) Losses from the seed bank of *Mimosa pigra*: soil microorganisms versus temperature fluctuations. *Journal of Applied Ecology*, **30**, 654–660.

Makkar, H.P.S. (2003) *Chapter 1: Chemical, Protein Precipitation and Bioassays for Tannins in Quantification of Tannins in Tree and Shrub Foliage – A Laboratory Manual*. Kluwer Academic Press, Dordrecht, the Netherlands.

Masaki, T. & Nakashizuka, T. (2002) Seedling demography of *Swida controversa*: effect of light and distance to conspecifics. *Ecology*, **83**, 3497–3507.

Masaki, T., Shibata, M. & Nakashizuka, T. (1998) The seed bank dynamics of *Cornus controversa* and their role in regeneration. *Seed Science Research*, **8**, 53–63.

Maude, R.B. (1996) *Seedborne Diseases and Their Control: Principles and Practice*. CAB International Press, Wallingford, UK.

Milton, K. (1991) Leaf change and fruit production in 6 neotropical Moraceae species. *Journal of Ecology*, **79**, 1–26.

Murray, K.G. & Garcia, J.M. (2002) Contributions of seed dispersal and demography to recruitment limitation on a Costa Rican could forest. *Seed Dispersal and Frugivory; Ecology, Evolution, and Conservation* (eds D.J. Levey, W.R. Silva & M. Galetti), pp. 323–338. CAB International Press, Wallingford, UK.

Neergaard, P. (1977) *Seed Pathology*, Vol. 1. Halsted Press, New York, NY, USA.

Neher, D.A., Augspurger, C.K. & Wilkinson, H.T. (1988) Influence of age structure of plant populations on damping-off epidemics. *Oecologia*, **74**, 419–424.

O'Hanlon-Manners, D.L. & Kotanen, P.M. (2004) Evidence that fungal pathogens inhibit recruitment of a shade-intolerant tree, White Birch (*Betula papyrifera*), in understory habitats. *Oecologia*, **140**, 650–653.

Orrock, J.L. & Damschen, E.I. (2005) Fungi-mediated mortality of seeds of two old-field plant species. *Journal of the Torrey Botanical Society*, **132**, 613–617.

Parker, I.M. & Gilbert, G.S. (2004) The evolutionary ecology of novel plant–pathogen interactions. *Annual Review of Ecology, Evolution and Systematics*, **35**, 675–700.

Pearson, T.R.H., Burslem, D.F.R.P., Mullins, C.E. & Dalling, J.W. (2002) Germination ecology of neotropical pioneers: interacting effects of environmental conditions and seed size. *Ecology*, **83**, 2798–2807.

Porter, L.J., Hrstich, L.N. & Chan, B.C. (1986) The conversion of procyanidins and prodelphinidins to cyanidin and delphinidin. *Phytochemistry*, **25**, 223–230.

Scalbert, A. (1991) Antimicrobial properties of tannins. *Phytochemistry*, **30**, 3875–3883.

Schafer, M. & Kotanen, P.M. (2003) The influence of soil moisture on losses of buried seeds to fungi. *Acta Oecologica*, **24**, 255–263.

Schafer, M. & Kotanen, P.M. (2004) Impacts of naturally-occurring soil fungi on seeds of meadow plants. *Plant Ecology*, **175**, 19–35.

Siemens, D.H., Johnson, C.D. & Ribardo, K.J. (1992) Alternative seed defense mechanisms in congeneric plants. *Ecology*, **73**, 2152–2166.

U'Ren, J.M., Dalling, J.W., Gallery, R.E., Maddison, D.R., Davis, E.C., Gibson, C.M. & Arnold, A.E. (2009) Diversity, phylogenetic relationships, and evolutionary origins of fungi associated with seeds of a neotropical pioneer tree. *Mycological Research*, **113**, 432–449.

Valencia, R., Foster, R.B., Villa, G., Condit, R., Svenning, J.C., Hernandez, C., Romoleroux, K., Losos, E., Magard, E. & Balslev, H. (2004) Tree species distributions and local habitat variation in the Amazon: large forest plot in eastern Ecuador. *Journal of Ecology*, **92**, 214–229.

Vázquez -Yanes, C. & Orozco-Segovia, A. (1993) Patterns of seed longevity and germination in the tropical rainforest. *Annual Review of Ecology and Systematics*, **24**, 69–87.

Vázquez-Yanes, C. & Smith, H. (1982) Phytochrome control of seed germination in the tropical rain forest pioneer trees *Cecropia obtusifolia* and *Piper auritum* and its ecological significance. *New Phytologist*, **92**, 477–485.

Veldman, J.W., Murray, K.G., Hull, A.L., Garcia-C, J.M., Mungall, W.S., Rotman, G.B., Plosz, M.P. & McNamara, L.K. (2007) Chemical defense and the persistence of pioneer plant seeds in the soil of a tropical cloud forest. *Biotropica*, **39**, 87–93.

Welbaum, G.E. (2006) Natural defense mechanisms in seeds. *Handbook of Seed Science and Technology*. (ed. A.S. Basra), pp. 451–464. Haworth Press, Binghamton, NY, USA.

Wills, C., Condit, R., Foster, R.B. & Hubbell, S.P. (1997) Strong density- and diversity-related effects help to maintain tree species diversity in a neotropical forest. *Proceedings of National Academy of Sciences of the United States of America*, **94**, 1252–1257.

Wright, S.J., Muller-Landau, H.C., Calderon, O. & Hernandez, A. (2005) Annual and spatial variation in seedfall and seedling recruitment in a neotropical forest. *Ecology*, **86**, 848–860.

Wulff, R.D. (1986) Seed size variation in *Desmodium paniculatum*: II. Effects on seedling growth and physiological performance. *Journal of Ecology*, **74**, 99–114.

Received 3 April 2009; accepted 11 September 2009
Handling Editor: Kyle Harms