INSIGHTS

The Role of Natural Enemies in the Germination and Establishment of *Pachira* (Malvaceae) Trees in the Peruvian Amazon

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ABSTRACT

Seeds from *Pachira brevipes* from white-sand forest and *Pachira insignis* from clay forest were placed in a reciprocal transplant experiment that manipulated herbivore presence. *Pachira insignis* experienced 80 percent leaf loss in both habitats from herbivores, causing 79 percent mortality in white-sand compared with 47 percent in clay in just 3 mo. *Pachira brevipes* suffered pathogen attack in both habitats that killed 90 percent of its establishing seedlings. Natural enemies thus strongly influence seedling recruitment of these two *Pachira* species in these forests and appear to have the largest impact within the seedlings' first few months.

Abstract in Spanish is available at http://www.blackwell-synergy.com/loi/btp.

Key words: Allpahuayo-Mishana National Reserve; clay soil; herbivory; reciprocal transplant experiment; seed predation; seedling recruitment; tropical rain forest; white sand.

THE SPATIAL DISTRIBUTION OF PLANT RECRUITMENT is influenced by both biotic and abiotic factors. When there are nonrandom distribution patterns in different plant species across an environmental gradient, it is important to consider all life stages that may be contributing to such habitat specialization (Gómez-Aparicio 2008). First, seed dispersal may not occur equally to all habitat types due to the behavior of the animal dispersal agent (Wenny 2000) or physical factors (*i.e.*, seeds adapted for dispersal by water will not often arrive on a hilltop). Second, although seeds may arrive in many habitats, seed predators may cause more mortality in some habitat types than others (Louda 1982). Third, there may be differential germination or establishment in different habitats due to abiotic factors such as light (Pearson *et al.* 2003) or H/Al ratios (Proctor 1999), or biotic factors such as leaf litter (Daws *et al.* 2005) or mycorrhizae (Smith & Read 2008). Finally, herbivores or diseases may cause habitat-specific patterns of mortality after a seedling establishes, either because different species of natural enemies live in different habitats (Parker & Root 1981) or because enemies interact with existing resource availability differences of the habitats, causing differential impact and mortality (Fine *et al.* 2004). For each of these life stages, if particular biotic or abiotic factors are to be implicated in driving habitat specialization by plants, then these factors must be demonstrated to contribute to differential success for a plant species with respect to habitat type.

Recently, Fine *et al.* (2004) reported that herbivores interacted with soil factors to promote habitat specialization by trees in Amazonian white-sand and clay forests. Species from clay forests outperformed white-sand specialists in both habitats when protected against herbivores. When unprotected, however, white-sand specialists outperformed clay specialists in white-sand forests, and clay specialists outperformed white-sand specialists in clay forests (Fine

et al. 2004). We interpreted these results as evidence for herbivores promoting habitat specialization in these forests, as well as support for the idea of an evolutionary trade-off between growth rate and defense that is associated with habitat specialization across a gradient in resource availability (Fine *et al.* 2006). This study was performed, like the great majority of reciprocal transplant experiments, on established seedlings, that were protected in an enclosed, herbivore-free cage for a few weeks to a few months before being transplanted into the experiment and initiating measurements. Yet, while the experiment provided convincing evidence about the role of herbivores and their interaction with soil type influencing performance and mortality of seedlings, it ignored the earlier life stages in these species, which are also likely to influence habitat specialization.

Here, we report a subsequent experiment using seeds of two habitat specialist trees from the genus *Pachira* Aubl. (Malvaceae), one from white-sand forest and one from *terra firme* clay soils. We placed the seeds of these two species in the same experimental setup as the reciprocal transplant experiment for seedlings in Fine *et al.* (2004) to ask the following questions: (1) To what degree does seed predation influence habitat specialization? (2) Can habitat specialist species germinate and establish in the other habitat type? (3) What is the role of herbivores and diseases in the first weeks of a seedlings life? Do they interact with habitat type? (4) How do herbivory and mortality rates for very young seedlings in these species compare with the older seedlings in the original transplant experiment?

The focuses of this study are *Pachira brevipes* (A. Robyns) W.S. Alverson and *Pachira insignis* (Sw.) Sw. ex Savigny. *Pachira insignis* is widely distributed throughout the Amazon basin, whereas *P. brevipes* is restricted to Peru and Colombia. In Peru, *P. insignis* is found on *terra firme* clay and brown-sand soils and *P. brevipes* is known only from white-sand forests (Vásquez Martinez 1994, Fine *et al.* 2010). *Pachira brevipes* is the most common tree species in a recent inventory of 16 white-sand plots from seven different

locations in the Peruvian Amazon (Fine *et al.* 2010). Both species are canopy trees with palmately compound leaves. *Pachira brevipes* produces small seeds *ca* 1 cm in diam attached to cottony kapok fibers that aid dispersal by wind. *Pachira insignis* produces larger edible seeds, 4–5 cm in diam that fall to the ground that are eaten and presumably dispersed by scatter-hoarding vertebrates.

In May 2001, we built 22 control and 22 herbivore exclosures ($3 \times 3 \times 2$ m); half were located in terra firme forest in one site on clay soils and half in one site in white-sand forest in the Allpahuayo-Mishana National Reserve near Iquitos, Peru ($3°57'$ S, $73°26'$ W) (Fine *et al.* 2004). White-sand forest in the Peruvian Amazon occurs as small habitat islands, immediately adjacent to other soil types. These habitats are rare, and the 'islands' are generally 10–100 km distant from one another (Fine *et al.* 2010). Differences between white-sand and clay soils are extreme: available nitrate and ammonium were an order of magnitude higher in clay than white-sand forest (Fine *et al.* 2006). Exclosures were covered with a green 1-mm nylon mesh that extended to ground level. Controls had the same nylon netting on the roof of the cage to shade the seedlings to a similar degree as the exclosures. To ensure that controls were experiencing attack from different individual insects, all exclosures and controls were 20–50 m apart from one another, randomly intermixed and distributed over 2 ha for each soil type.

In February 2003, sufficient numbers of seeds were found for *P. brevipes* and *P. insignis* to test for the relative role of natural enemies and soil factors at the germination and seedling establishment phase. For *P. brevipes*, seeds were collected from fruits from three different mother trees. The seeds were removed from ripe capsules that were still attached to the tree. For *P. insignis*, seeds were collected from fruits that had fallen to the ground beneath three mother trees. Seeds from both species were not surface sterilized and were immediately moved to the experimental locations. In each of the 44 control and exclosures, three seeds from each of the two species were placed next to marked sticks. These seeds were visited every 2 wk for 3 mo. For each seed, we recorded whether it died, germinated or was eaten by a seed predator. Subsequently, for each germinated seedling, data were collected on the number of leaves produced, the number of leaflets removed by herbivores and if the seedling died. Percent germination, leaf herbivory (number of leaflets consumed/total number of leaflets produced) and mortality were calculated for each of the control and exclosures. For each species, we examined the response of each of the three response variables using generalized linear mixed models to account for the random effect of plot while examining the effects of habitat and exclosure treatment. For germination and survival, which are binary response variables, we ran a model using a binomial family distribution. Herbivory rates were strongly skewed to values with no herbivory, so we created a dummy binary variable indicating if plants lost any leaflet area to herbivory during the course of the experiment, and modeled this in a parallel framework as germination and survival rates. All analyses were conducted in the lme4 package (Bates & Maechler 2010) of the R 2.10 statistical platform (R core development team 2010).

Neither species suffered a significant amount of seed predation by animals, with *P. brevipes* being completely ignored (0% seed predation in both habitats), and *P. insignis* experiencing very low rates in both habitat types (2–7%, Table 1). The latter result was surprising, as *P. insignis* is thought to be adapted for seed dispersal by rodents because it produces large, sweet nuts that fall to the ground in clusters (personal observation and consumption by both authors). Emmons (1984) reported extremely low rodent densities in white-sand forests in Mishana (a few km away from our study site), but high diversity and abundances in nearby forests of similar soil types to our clay forest site. Perhaps these seeds require larger densities to attract vertebrates that will both predate and scatter-hoard the seeds. The seed removal rate that we report here is much lower than several other studies published from Neotropical moist forests (reviewed by Crawley 1992, Wenny 2000). For example, Wenny (2000) reported over 99 percent seed predation of *Ocotea* (Lauraceae) seeds in Monteverde, Costa Rica.

Neither the white-sand nor clay specialist *Pachira* demonstrated statistically significant habitat-specific germination patterns (Table 1). *Pachira insignis*, the clay specialist, germinated about 90 percent of its seeds in both habitats, while *P. brevipes*, the white-sand specialist germinated a third of its seeds in clay habitat, and almost one half of its seeds in white-sand habitat (GLM z-test, $P > 0.2$). Almost all of the *P. brevipes* seedlings died within 3 mo in both habitats, whether protected from herbivores or not (Table 1). Those seedlings that did establish died soon after producing their first leaves, and appeared to succumb to a disease affecting their roots. There was no extended drought during the experiment, and the seedlings died with their leaves intact, with full turgor pressure. They fell over, and subsequent examination of their roots revealed a soft, mushy texture. Although the seeds were not surface sterilized before planting, we believe that contamination from the source is extremely unlikely given that the seeds were collected from fruits still attached to the mother tree and then carried to the experimental site in plastic bags. It is interesting that these symptoms were not noticed on any of the *P. insignis* seedlings, considering the close proximity of all experimentally planted seeds. *Pachira brevipes* is adapted for wind-dispersal and is small-seeded like many white-sand specialist trees (Macedo & Prance 1987) and this may predispose this species to high susceptibility to fungal attack (Nakamura *et al.* 1995). In general, small-seeded species have been found to be more vulnerable to establishment-related death than large seeded species because of their relatively smaller root and shoot diameters (Daws *et al.* 2007). All *P. brevipes* seedlings suffered similar mortality rates irrespective of habitat type in this experiment. Future studies could culture the pathogen from dying seedlings and then use it in an experimental manipulation in order to evaluate the role of this pathogen in habitat specialization of *Pachira*.

In contrast to *P. brevipes*, the clay specialist *P. insignis* established and grew well in both habitats. Subsequently, after the seedlings flushed out several leaves, there was a significant effect of herbivore protection on herbivory rates (GLM z-test, $P < 0.01$) and mortality (GLM z-test, $P < 0.024$, Fig. 1). Unprotected seedlings suffered more than 80 percent herbivory in both white-sand and clay habitats, and this attack resulted in 47 percent mortality in clay habitat, and 79 percent mortality in white-sand forest (Fig. 1). When protected from herbivores, *P. insignis* seedlings had

TABLE 1. *Results from germination, and establishment experiment for* Pachira *species seeds placed in the experimental exclosures (protected) and controls (unprotected). Mortality during establishment refers to death before producing new postcotyledon leaves. There was no significant effect of habitat or treatment in germination or establishment-related mortality in either species (GLM z-tests).*

Species	Variable	Clay habitat	White-sand habitat
Pachira insignis	Percent germinated	88	92
	Percent killed by seed predators		
	Protected	0	0
	Unprotected	7	2
	Percent mortality during establishment		
	Protected	0	0
	Unprotected	0	0
Pachira brevipes	Percent germinated	33	49
	Percent killed by seed predators		
	Protected	0	0
	Unprotected	0	0
	Percent mortality during establishment		
	Protected	93	85
	Unprotected	83	80

very high survival rates (93–97%) on both clay and white-sand habitats (Fig. 1).

These results extend our understanding of habitat specialization in these forests in two significant ways. First, they show that postdispersal seed predation, germination and establishment are not important factors contributing to differential success of *P. insignis* across white-sand and clay habitats. Second, they show that herbivores can cause a very large effect in a very short window of time. In less than 3 mo, most of the seedlings' leaf tissue was consumed, causing extremely high mortality rates. The mortality rates

experienced by young seedlings are almost ten times greater than reported in the seedling experiment of Fine *et al.* (2004) (Table 2). Other reciprocal transplant experiments between habitats that have reported much lower herbivory and much lower mortality could be underestimating the effect of natural enemies by not including seeds and young seedlings in their experiment. For example, Eichhorn *et al.* (2006) report young leaf herbivory at 1.96–4.8 percent/mo for older seedlings of five species of habitat specialist dipterocarps in Malaysian Borneo over a 2-yr period. These seedlings were at least 3 yr old by time of transplantation (Baltzer *et al.* 2005).
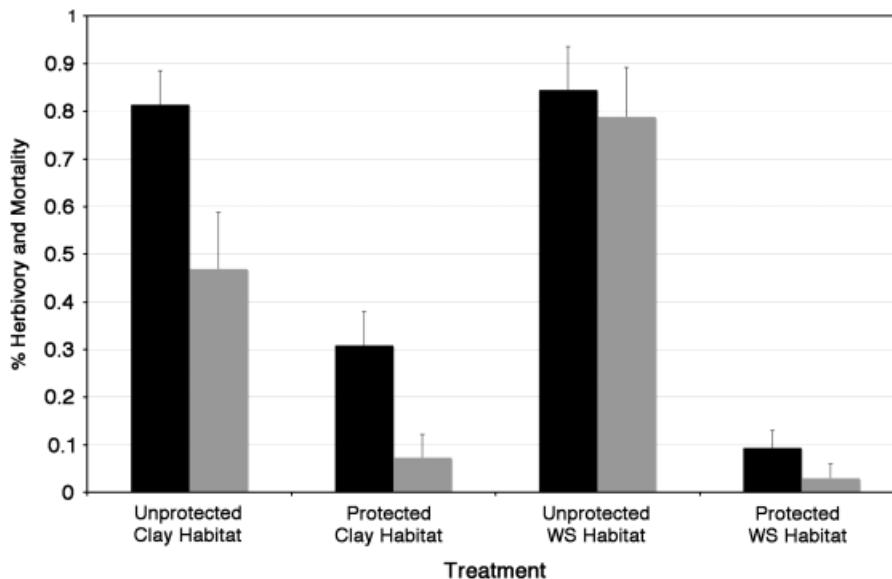

FIGURE 1. Post-establishment herbivory and mortality rates for *Pachira insignis* over the 3 mo experiment. Herbivory rates are represented by black bars and mortality rates are represented by gray bars. Both bars include +1SE.

TABLE 2. *Comparison of mortality rates of* Pachira insignis *between the two experiments. There were a total of 52 seedlings of* P. insignis *planted in the 2000–2003 experiment and a total of 112 seedlings of* P. insignis *planted in the 2003 experiment. Mortality rates were calculated per control and exclosure and averaged for each of the 11 replicates for each soil type. Data from the 2000–2003 experiment comes from Fine* et al. *(2004).*

Experiment	Mortality rate in unprotected clay habitat (% mortality per mo)	Mortality rate in protected clay habitat (% mortality per mo)	Mortality rate in unprotected white-sand habitat (% mortality per mo)	Mortality rate in protected white-sand habitat (% mortality per mo)
2000–2003 Seedlings 6–27 mo	1.8	0	2.0	1.1
2003 Seedlings 0–3 mo	15.7	2.3	26.3	1.0

Moreover, the results demonstrate that for *P. insignis*, seedlings are much more vulnerable to herbivore attack in their first 3 mo compared with seedlings from 6 to 27 mo (Table 2). Boege and Marquis (2005) reviewed studies measuring herbivory at different plant life stages and concluded that defensive capabilities are quite dynamic throughout a plant's ontogeny. They hypothesized that cotyledons should be relatively highly defended, as the plants should be able to use stored resources from the seed to produce highly defended first leaves and that the defense level should drop as seedlings experience a shaded understory and will have access to very few resources to produce defenses for subsequent leaves. They further hypothesized that saplings should have higher defense levels than seedlings, because saplings will have a larger resource capture ability (roots, photosynthetic shoots and leaves). Our findings agree with this hypothesis, as those *P. insignis* seedlings that were killed by herbivores all had produced at least three leaves (postcotyledon) before they began to be attacked. Comparing the mortality rates to the older seedlings (Table 2), however, suggests that the amount of defenses allocated by seedlings is even more dynamic than posited by Boege and Marquis (2005), and that increases in defense levels can occur within the first months of life, while plants are still at the seedling stage. For *P. insignis*, herbivory and mortality rates were substantially higher for 3-mo-old seedlings than for older seedlings in the first experiment (Table 2). As far as we know, this is the first field reciprocal transplant of any plant species that compares herbivore-related mortality for seedlings from different age classes. We acknowledge, however, that the seeds in this experiment were planted after the first seedling experiment was concluded, and seasonal or interannual differences in climate, herbivore abundance or other factors could have influenced the results shown in Table 2. However, Iquitos does not experience seasonality with regards to temperature or rainfall (Marengo 1998), and there were no severe droughts during either experiment. Our mortality results are very similar to a recent study where seeds were placed in on the forest floor with wire cages that protected them from vertebrates and seedlings followed for 5 yr in a French Guianan rain forest (Baraloto *et al*. 2005). For seven out of eight species, the authors reported heavy mortality within the first 4 mo (40–88%) that leveled off afterwards (Baraloto *et al*. 2005).

Some previous studies investigating habitat association of tropical tree seedlings have emphasized abiotic factors over herbivores and disease (*i.e.*, Proctor 1999, Baltzer *et al*. 2005, Luizão *et al*. 2007). The effect of natural enemies on plants can be difficult to measure because herbivores and diseases attack plants in spatially nonrandom patterns (Schupp 1988), may effect certain life stages to a greater degree than others (Gómez-Aparicio 2008), cause damage that is difficult to see or quantify (Massey *et al*. 2005) or vary in abundance from year to year (Carson & Root 2000). The best way to evaluate the relative importance of natural enemies in habitat specialization is to include, to the extent possible, all life stages of a plant species from seed to juvenile, and to monitor herbivore exclosures together with controls for long periods of time. Here, we found that biotic factors caused massive mortality in both species of habitat specialist *Pachira*, and this result was especially pronounced for extremely young seedlings. For the white-sand specialist *P. brevipes*, most seedlings appeared to die from pathogen attack during the establishment phase. For *P. insignis*, herbivory rates were extremely high in both habitats, causing higher mortality rates in white-sand habitats compared with clay forests. We believe that the mechanism of differential mortality in white-sand forest for *P. insignis* relates to the growth-defense trade-off and the difficulty of replacing leaf tissue in the impoverished white-sand soils (Fine *et al*. 2006). This interaction of herbivore attack with soil resources promotes habitat specialization for *P. insignis*, and the rates of herbivore attack and mortality were almost ten times greater than those reported for older seedlings, suggesting that the growth-defense trade-off's effect on habitat specialization may be particularly acute for the youngest seedlings.

ACKNOWLEDGMENTS

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