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SHORT COMMUNICATION

Seed predation, pathogens and germination in primary vs. secondary cloud forest at Maquipucuna Reserve, Ecuador

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Abstract: Because the fate of the seed rain has long been shown to be critical to understanding forest recruitment and regeneration, seed predation, seed pathogens and germination among different species was examined in primary (closed-canopy vs. tree-fall gap) and in secondary (banana vs. sugarcane vs. seeded pasture) cloud forest at Maquipucuna Reserve, Ecuador. I found (1) seed predation took more seeds than either seed pathogenic disease or germination for all tree seed species and in both forests, where the level of seed loss to predation was greatest in the closed-canopy primary forest, second largest in the tree-fall gaps and less in recovering banana, sugarcane and pasture; (2) for pathogens these trends were reversed; and (3) most seeds, that were not taken by predators or pathogens, germinated. *Cecropia* sp. seeds in the tree-fall gaps and *Otoba gordoniifolia* seeds in both closed-canopy forest and tree-fall gaps were the most significantly different among all treatments in primary forest and *Solanum ovalifolia* seeds in banana fields and *Piper aduncum* in all fields were the most significantly different among all treatments in secondary forest. I conclude that forests may recover faster after human disturbance (here agriculture) than after natural disturbances (here tree-fall).

Key Words: banana, LTER, pasture, sugarcane

Cloud forests exist in mountains (500 m to 3000 m asl) around the world (Grubb 1977). Whereas cloud forests may be disturbed by a variety of natural processes, such as tree-fall, landslides (Myster & Sarmiento 1998) and high-wind events, the cutting down of wooded areas for agriculture and pasture is its major cause of deforestation (Muniz-Castro *et al.* 2015). Consequently comparisons between undisturbed primary cloud forest and secondary cloud forest recovering from agriculture are critical to understanding current and future cloud forests. Therefore I expanded on past recruitment studies done in cloud forests (see review in Myster 2004a) by examining three main sources of variation in the workings of the seed recruitment processes of predation, pathogenic attack and germination (1) between closed-canopy primary cloud forest and its naturally occurring tree-fall gaps; (2) among recovering banana fields, recovering sugarcane fields, and seeded pastures no longer being grazed in secondary cloud forest; and (3) among tree genera or species commonly found in both forests.

The study site was the Maquipucuna Reserve, Ecuador (0°05'N, 78°37'W; www.maqui.org; Rhoades & Coleman 1999, Rhoades *et al.* 1998, Sarmiento 1997) which lies between 1200 and 1800 m asl. The Reserve is a patchwork of primary cloud forest (Edmisten 1970) and secondary cloud forest recovering from human impacts, mainly cultivation of sugarcane and banana, and pasture seeded with the grass *Setaria sphacelata* which is native to Africa (Sarmiento 1997) but no longer being grazed. Primary cloud forest was used in the experiment and new data from replicated field plots, set up in 1997 in secondary cloud forest with the same protocol (Myster 2004b, 2012) are also related here. Ten primary cloud forest areas were randomly selected in May 2014, five of which had recent, average-sized (all between 100–300 m^2 in area: Brokaw 1982) tree-fall gaps in them. Ripe fruits were first hand-collected (using gloves) locally from one individual of one common early-successional tree (*Cecropia* sp., bat/bird dispersed), and from four common mid-successional/subcanopy

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Table 1. The fate of seeds expressed as a percentage (mean ± SE) of the total seeds put out in closed-canopy forest/in tree-fall gaps. The experiment was performed in primary cloud forest, Maquipucuna reserve, Ecuador.

Species	% lost to predators	% lost to pathogens	% germinated	% other
Cecropia sp.	88 ± 1/70 ± 2	8 ± 2/19 ± 1	4 ± 1/6 ± 1	0 ± 1/5 ± 2
Ardisia websteri	85 ± 1/81 ± 1	4 ± 1/10 ± 1	11 ± 2/9 ± 1	0 ± 1/0 ± 1
Prestoea acuminata	80 ± 3/88 ± 2	3 ± 2/5 ± 2	10 ± 2/6 ± 2	7 ± 2/0 ± 1
Ficus sp.	82 ± 2/92 ± 1	5 ± 1/3 ± 3	8 ± 1/4 ± 3	5 ± 1/1 ± 1
Otoba gordoniifolia	70 ± 1/68 ± 1	4 ± 2/10 ± 3	20 ± 1/10 ± 2	6 ± 2/12 ± 1

trees (*Ardisia websteri*, *Prestoea acuminata*, *Ficus* sp., *Otoba gordoniifolia*, all bird dispersed) with obviously damaged and/or empty seeds discarded in the field. Seeds were then hand-sorted (again using gloves) in the laboratory, visually inspected for damage under a dissecting microscope, and then floated to further exclude non-viable seeds. Finally 10 seeds of each of the five test species were placed separately in sets of five plastic 9-cm diameter Petri dishes spaced 30 cm apart in the centre of each closed-canopy forest area and each tree-fall-gap area, for a total of 50 dishes and 500 seeds. Heavy plastic seed mimics were also placed in each dish to determine whether or not seeds were removed by a biotic agent (e.g. insects, mammals) or an abiotic agent (e.g. wind, rain).

After 2 wk in the field seed loss was scored in each dish, which was assumed to be due to action by an animal – not wind or splashing rain – because (1) evidence of animals was observed while collecting these data (e.g. chewed seeds and husks, small-mammal faeces) and (2) duplicate plastic seed mimics were not lost. In addition the assumption was made that seeds carried away were eaten, and/or rendered non-viable in some other way associated with the action of an animal agent (Notman & Gorchov 2001), and so did not germinate later after being removed by an animal. This assumption has been discussed in the literature for several years (Myster 2007, 2014) but to date no study has produced statistically significant results to question its validity. Indeed attempts to track seeds in the field after animals take them – using, for example, fishing line glued to seeds or tagging seeds using radioactive isotopes, magnets and fluorescent dyes – may lead to experimental side effects of their own, which has not been discounted (Forget *et al.* 1999). Only when recruitment after seed removal by animals has been shown to a significant part of a plant's recruitment and a seed-following methodology has been proved to be non-invasive, should this assumption be reexamined.

The remaining seeds (not removed) were collected for incubation, with any empty seed hulls discarded, and incubated in a Maquipucuna shadehouse on moist paper in sealed plastic Petri dishes. After 3 wk, seeds that germinated, seeds that did not germinate and had extensive fungal infection (lost to pathogenic attack where contact with the soil is not necessary for infection: Myster 2004b), and other seeds were scored after viewing under a dissecting microscope. Two-way analysis of variance (ANOVA: SAS 1985) was performed separately for all seed processes (closed-canopy forest vs. tree-fall, among species). In all tests if significance was found, means tests were conducted with the Tukey procedure (SAS 1985). Prior to statistical analysis, all data were examined and found to be normally distributed. Results need to be viewed with the understanding that the primary cloud forest study and the secondary cloud forest study were done in different years and only have one genus in common among the seeds used in the experiments.

Seed predation took more seeds than either seed pathogenic disease or germination for all tree seed species and in both primary cloud forest (Table 1) and secondary cloud forest (Table 2). The level of seed loss to predation was greatest in the closed-canopy primary forest (mean loss = 81%) and second largest in the tree-fall gaps found in closed-canopy primary forest (80%). Losses due to predation in secondary forest were less than those in primary forest, with recovering banana fields showing the largest losses (67%), followed by sugarcane (65%) and then pasture (61%). For pathogens, losses in the closed-canopy primary forest were smaller (5%) than in the tree-fall gaps (9%). Seed losses to pathogens in secondary forest were greater than those in primary forest: banana fields (19%), sugarcane fields (19%) and pastures (19%). Finally germinated seeds in the closed-canopy primary forest were greater (10%) then in the tree-fall gaps (7%) and germination in secondary forest was greater than in primary forest: banana fields (11%), sugarcane fields (10%) and pastures (11%).

Analysis of variance showed that in the primary cloud forest seed predation was significantly different between closed-canopy and tree-fall gap (F = 5.2, P = 0.005), among the test species (F = 3.1, P = 0.04) and in the interaction between these two main effects (F = 4.2, P = 0.02). For seeds lost to pathogens and germinated seeds, however, only pathogenic seed losses were significant and then only among seed test species (F = 3.7, P = 0.03). In the secondary cloud forests, ANOVA showed that seed predation was again significantly different for both main effects (banana vs. sugarcane vs. pasture F = 6.6,

Table 2. The fate of seeds expressed as a percentage (mean ± SE) of the total seeds put out in fields recovering from banana cultivation/in fields recovering from sugarcane cultivation/in fields recovering after use as planted pasture. The experiment was performed in secondary cloud forest, Maquipucuna reserve, Ecuador.

Species	% lost to predators	% lost to pathogens	% germinated	% other
Cecropia monostachya	66 ± 1/67 ± 2/50 ± 1	24 ± 2/25 ± 1/29 ± 3	10 ± 2/6 ± 1/10 ± 1	0 ± 1/2 ± 1/11 ± 1
Piper aduncum	50 ± 1/50 ± 2/49 ± 2	30 ± 1/30 ± 1/29 ± 2	11 ± 3/10 ± 1/12 ± 1	9 ± 1/10 ± 2/0 ± 1
Cestrum megalophyllum	74 ± 2/69 ± 1/71 ± 2	13 ± 1/11 ± 2/10 ± 1	10 ± 2/9 ± 2/9 ± 1	3 ± 1/11 ± 3/9 ± 1
Solanum ovalifolium	77 ± 2/75 ± 1/71 ± 3	9 ± 1/9 ± 1/8 ± 3	14 ± 1/14 ± 2/13 ± 1	0 ± 1/2 ± 2/7 ± 2

P = 0.003, among the test species F = 2.8, P = 0.04) and for the interaction term (F = 3.2, P = 0.03). The significant interaction terms (seed predation for both primary and secondary forests) are most revealing for the ANOVAs (SAS 1985) and therefore were explored further. *Cecropia* sp. seeds in the tree-fall gaps and *Otoba gordoniifolia* seeds in both closed-canopy forest and tree-fall gap, were the most significantly different among all treatments (*Cecropia* sp. had 70% loss, *Otoba gordoniifolia* had 70% loss and 68% loss, respectively). *Solanum ovalifolium* seeds in banana fields (had the highest loss 77%) and *Piper aduncum* in all fields (had the lowest loss 50% in banana fields, 50% in sugarcane fields, 49% in seeded pastures) were the most significantly different among all treatments.

Predation was, once again, the major seed mechanism of forest recruitment (see reviews in Myster 2004a, 2007) across both primary (closed-canopy, tree-fall gap) and secondary (banana, sugarcane, pasture) forests. After predation, results show that pathogens could play an important role in recruitment and that most remaining seeds germinated (Myster 2003, Myster & Everham 1999). Taken together, results suggest that it is the variation in how seed predation works (e.g. among species, under litter, at different times of the year; Myster 2004b) that is a key to understanding recruitment and regeneration in cloud forest. When we compare the results to other secondary Neotropical forests we find much in common: (1) over 75% loss of seed to predators (more by insects in mature Peru forest and more by mammals in old fields; Notman & Gorchov 2001); (2) the same trends (seed loss due to predation > that due to pathogens > seeds that germinated) in Puerto Rico pasture, banana, sugarcane fields (Myster 2004a), and in Puerto Rico coffee fields and pasture (50–75% predation, 20–45% pathogens, germination 15–35% depending on species; Myster 2003); and (3) after clear-cutting for logging in Peru several tree species had a reduction of seed predation from the forest into the clear-cut (50–90% losses; Notman *et al.* 1996).

Other Neotropic forest studies suggest further complexity and future experiments by showing that (1) larger seeds get taken by pathogens more than smaller seeds, germination was approximately 43% and pathogen loss was up to 75% depending on species (Pringle *et al.* 2007); (2) secondary dispersal is low (Culot *et al.* 2009, Parolin *et al.* 2004); (3) seed predation was reduced under litter, especially under thick litter (Cintra 1997); and (4) after bat defecation seed were eaten at a rate of 8% wk^{-1} with possible satiation (Romo *et al.* 2004). My own results in other Neotropic forests (Myster 2014, author, unpubl. data) suggest that (1) seed predation may be reduced to similar levels more by loss of soil fertility (i.e. in palm forest) than by increased flooding (i.e. in igapó forests); (2) for pathogens, standing water in palm forest leads to the greatest losses, but increased flooding in igapó forests can also lead to increasing loss of seeds to pathogens; and (3) most seeds germinated if they could escape predators and pathogens (Myster & Everham 1999). The fact that more seeds survived in the secondary forest, compared with other treatments, may be because predators avoid open spaces (Myster 2004b) and suggests that human disturbance (after agriculture) may accelerate the natural regeneration processes (after tree-fall) in this forest making it more dynamic.

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LITERATURE CITED

BROKAW, N. V. L. 1982. The definition of treefall gap and its effect on measures of forest dynamics. *Biotropica* 11:158–160.

CINTRA, R. 1997. Leaf litter effects on seed and seedling predators of the palm *Astrocaryum murumura* and the legume tree *Dipteryx micrantha* in Amazonian forest. *Journal of Tropical Ecology* 13:709–725.

CULOT, L., HUYNEN, M., GERARD, P. & HEYMANN, E. W. 2009. Short-term post-dispersal fate of seeds defecated by two small primate species (*Saguinus mystax* and *S. fuscicollis*) in the Amazonian forest of Peru. *Journal of Tropical Ecology* 25:229–238.

EDMISTEN, J. 1970. Some autoecological studies of *Ormosia krugii*. Pp. 291–298 in Odum, H. T. & Pigeon, R. F. (eds.). *A tropical rain forest*. National Technical Information Service, Springfield.

FORGET, P. M., KITAJIMA, K. & FOSTER, R. B. 1999. Pre- and post-dispersal seed predation in *Tachigali versicolor* (Caesalpiniaceae): effects of timing of fruiting and variation among trees. *Journal of Tropical Ecology* 15:61–81.

GRUBB, P. J. 1977. Control of forest growth and distribution on wet tropical mountains, with special reference to mineral nutrition. *Annual Review of Ecology and Systematics* 8:38–107.

MUNIZ-CASTRO, M. A., WILLIAMS-LINERA, G. & BENITEZ-MALVIDO, J. 2015. Restoring montane cloud forest: establishment of three Fagaceae species in the old fields of central Veracruz, Mexico. *Restoration Ecology* 23:26–33.

MYSTER, R. W. 2003. Seed regeneration mechanisms over fine spatial scales on recovering coffee plantation and pasture in Puerto Rico. *Plant Ecology* 166:199–205.

MYSTER, R. W. 2004a. Post-agricultural invasion, establishment and growth of Neotropical trees. *The Botanical Review* 70:381–402.

MYSTER, R. W. 2004b. Regeneration filters in post-agricultural fields of Puerto Rico and Ecuador. *Plant Ecology* 172:199–209.

MYSTER, R. W. 2007. *Post-agricultural succession in the Neotropics*. Springer-Verlag, Berlin.

MYSTER, R. W. 2012. Long-term data from fields recovering after sugarcane, banana and pasture cultivation in Ecuador. *Dataset Papers in Ecology* 2013: Article ID 468973.

MYSTER, R. W. 2014. Interactive effects of flooding and treefall gap formation on *terra firme* forest seed and seedling mechanisms and tolerances in the Amazon. *Community Ecology* 15:212–221.

MYSTER, R. W. & EVERHAM, , E. M. EVERHAM 1999. Germination cues across the disturbance regime in the Puerto Rican rainforest. *Tropical Ecology* 40:89–98.

MYSTER, R. W. & SARMIENTO, F. O. 1998. Seed inputs to microsite patch recovery on Tropandean landslides in Ecuador. *Restoration Ecology* 6:35–43.

NOTMAN, E. & GORCHOV, D. L. 2001. Variation in post-dispersal seed predation in mature Peruvian lowland tropical forests and fallow agricultural sites. *Biotropica* 33:621–636.

NOTMAN, E., GORCHOV, D. L. & CORNEJO, F. 1996. Effect of distance, aggregation, and habitat on levels of seed predation for two mammal-dispersed Neotropical rain forest tree species. *Oecologia* 106:221–227.

PAROLIN, P., DESIMOBNE, O., HAASE, K., WALDHIOFF, D., ROTTENBERGER, S., KUHN, U., KESSELMEIER, J., KLEISS, B., SCHMIDT, W., PIEDADE, M. T. F. & JUNK, W. J. 2004. Central Amazonian floodplain forests: tree adaptations in a pulsing system. *The Botanical Review* 70:357–380.

PRINGLE, E. G., ALVAREZ-LOAYZU, P. & TERBORGH, J. 2007. Seed characteristics and susceptibility to pathogen attack in tree seeds of the Peruvian Amazon. *Plant Ecology* 193:211–222.

RHOADES, C. C. & Coleman, D. C. 1999. Nitrogen mineralization and nitrification following land conversion in montane Ecuador. *Soil Biology and Biochemistry* 31:1347–1354.

RHOADES, C. C., ECKERT, G. E. & COLEMAN, D. C. 1998. Effect of pasture trees on soil nitrogen and organic matter: implications for tropical montane forest restoration. *Restoration Ecology* 6:262–270.

ROMO, M., TUOMISTO, H. & LOISELLE, B. A. 2004. On the density-dependence of seed predators in *Dipteryx micrantha*, a bat-dispersed rain forest tree. *Oecologia* 140:76–85.

SARMIENTO, F. O. 1997. Arrested succession in pastures hinders regeneration of Tropandean forests and shreds mountain landscapes. *Environmental Conservation* 24:14–23.

SAS 1985. *User's guide: statistics*, Ver. 5. SAS Institute Inc., Cary. 956 pp.