SEED REMOVAL IN AN ATLANTIC FOREST FRAGMENT AND A RESTORATION SITE IN SOUTHERN BRAZIL[1]

REMOÇÃO DE SEMENTES EM UM FRAGMENTO DE FLORESTA ESTACIONAL SEMIDECIDUAL E EM UM PLANTIO DE RESTAURAÇÃO NO SUL DO BRASIL

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ABSTRACT – In ecological restoration, the loss of seeds can be a major constraint to the establishment of seedlings and thus for the continuity of the forest succession process. The aim of this study was to evaluate the removal/predation of seeds in an area being restored (6.5 years old). We introduced seeds of six native tree species (five non-pioneers and one pioneer) into the restoration site, and also in an adjacent forest fragment, used as a control. Seed removal was greater in the forest fragment (56%) than in the restoration site (16%) for the whole set of species analyzed, and was more frequent during the first 15 days after exposure. In the forest fragment, four of the six species had more than 65% of their seeds removed. In the restoration site, seed removal did not surpass 40% for a single species and was lower than 5% for two species. The proportion of damaged seeds did not differ between environments (3.6% in the native forest and 7.6% in the area being restored). Our results indicate that post-dispersal seed removal is not a barrier to the establishment of tree species in the restoration site, suggesting that the secondary succession can be constrained by factors related to seed germination and/or seedling establishment.

Keywords: ecological restoration; late succession species; limiting factors; seedling recruitment; seed predation.

RESUMO – Na restauração ecológica, a falta de sementes pode representar a maior barreira para os processos de regeneração natural e, assim, comprometer a continuidade dos processos sucessionais. O objetivo deste estudo foi avaliar a remoção/predação de sementes em uma área em restauração, aos seis anos e meio após o plantio de espécies arbóreas nativas. Introduzimos sementes de seis espécies arbóreas nativas (cinco não pioneiras e uma pioneira) na área em restauração e em um fragmento de floresta adjacente, utilizado como controle. A remoção de sementes foi maior na floresta nativa (56%) do que na área em restauração (16%) para o conjunto das espécies analisadas, sendo maior nos primeiros 15 dias de exposição. Na área em restauração, nenhuma espécie apresentou mais de 40% de remoção e duas espécies apresentaram menos de 5%. A proporção de sementes danificadas não diferiu entre os ambientes (3,5% no fragmento florestal e 7,6% no reflorestamento). Nossos resultados indicam que a remoção de sementes pós-dispersão não é uma barreira para a regeneração de espécies arbóreas na floresta em restauração. Fatores relacionados com as condições para germinação de sementes e/ou o estabelecimento das plântulas podem estar limitando a sucessão secundária.

Palavras-chave: restauração ecológica; espécies tardias; fatores limitantes; recrutamento de plântulas; predação de sementes.

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1 INTRODUCTION

Planting trees can facilitate forest succession on degraded environments through changes in microclimate, soil conditions and by improving structure and functionality of the ecosystem (Parrotta et al., 1997; Society for Ecological Restoration International Science and Policy Working Group – SER, 2004), thereby promoting seed germination and seedling establishment and growth (Guariguata and Ostertag, 2001). However, abiotic and biotic barriers may slow or even impede natural regeneration (Aide and Cavelier, 1994; Nepstad et al., 1996; Holl and Lulow, 1997; Medina, 1998; Benítez-Malvido and Lemus-Albor, 2005; Vander-Wall et al., 2005) especially in early restoration sites.

Many studies pointed that seed predation is a process of interspecific interaction that can influence the composition and structure of plant communities (e.g., Holl and Lulow, 1997; Doust, 2011). Seed predation is responsible for the high mortality rate of seeds in some species (Wenny, 2000; Garcia-Orth and Martínes-Ramos, 2008; Jones et al., 2008; Doust, 2011), mainly in the tropics, where most of the seeds are eaten by rodent predators (Cole, 2009; Vélez-Garcia and Pérez-Torres, 2010), which affects germination and seedling establishment (Molofsky and Fisher, 1993; Wenny, 2000; Jones et al., 2008).

Studies have shown that rates of seed predation may be modified when forests are altered (Aide and Cavelier, 1994; Nepstad et al., 1996; Wijdeven and Kuze, 2000; Baldissera and Ganade, 2005). Garwood (1989) suggested that the period that seeds remain in the soil is determined by the physiological properties of the seeds, environmental conditions, and the presence of predators and/or pathogens. Additionally, the seeds are more vulnerable to predation at sites with less litter, which are associated with changes in seed germination and seedling establishment, the microclimate, and the time of exposure to seed predators (Cintra, 1997). Potential predators of seeds that are characteristic of altered vegetation can invade forest edges and tree plantations (Olifiers et al., 2005). Other studies have found that seed predation by invertebrates, particularly ants, causes reduction in amount of availability of seeds for regeneration in tropical environments (Nepstad et al., 1996; Holl and Lulow, 1997). Limited food and seed rain in successional environments may also increase pressure on the propagules, resulting in greater mortality than in mature forests (Dirzo and Miranda, 1990).

Thus, seed predation may act as an ecological constraint to natural regeneration also in restored ecosystems and, in this context, the present study aimed to investigate if post-dispersal seed predation, as inferred by seed removal, can act as a barrier for natural regeneration of arboreal species in Atlantic Forest restoration sites. To achieve this, differences in seed predation rates between an restoration site and a native forest fragment used as reference were compared. Our main hypothesis was that seed predation in the restoration site would be higher than that in the forest fragment.

2 METHODS

2.1 Study Site

The study site is located at "Fazenda Congonhas" (22º59'S and 50º56'W; Figure 1) north of the Paraná state, southern Brazil. The restoration site occupies a riparian area of 11.8 ha, and the control site is an adjacent forest fragment covering 107.8 ha, both along the margins of Tibagi river. The forest fragment is a remnant of Seasonal Semi-deciduous Atlantic forest, surrounded by agriculture.

The restoration was done by planting seedlings of 25 native tree species in 2 x 3 m spacing. A high density (83%) of seedlings from pioneer and early successional species was used in order to provide early shade and suppress exotic grasses. The soil preparation was mechanized, without the addition of fertilizers and the weed control were done by manual and mechanized weeding for two years, without using herbicides. After five years, the restoration site was an open forest structure, with high litter mass, 73% of the forest basal area and 89% of forest canopy cover (Table 1). However, juvenile density (590 ind.ha^{-1}, from 10 cm to 1 m in height) was still low when compared to other restoration sites (Dias, 2011).

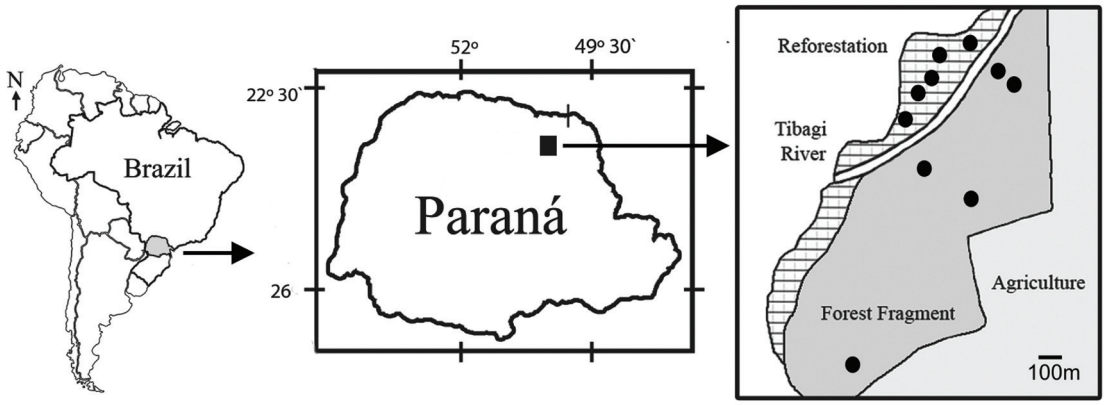

Figure 1. The study sites in the northern Paraná state, southern Brazil. In the right box the portion in gray indicates the forest fragment (107.8 ha) and the dashed area indicates the restoration site (11.8 ha). The black dots indicate the replicates of the seed removal experiment.

Figura 1. Locais do estudo no norte do Estado do Paraná, Sul do Brasil. Na caixa à direita, a porção em cinza corresponde ao fragmento florestal (107,8 ha), e a área hachurada, à área em restauração (11,8 ha). Os pontos pretos apontam a localização das réplicas do experimento de remoção de sementes.

Table 1. Features of the native forest fragment and the restoration site in the northern Paraná state, southern Brazil. Data was gathered by Suganuma (2008) five years after the seedlings were planted in the restoration site, and one year before the seed removal experiment assembly.

Tabela 1. Características do fragmento de floresta nativa e do reflorestamento com espécies nativas no norte do Estado do Paraná, Sul do Brasil. Os dados foram coletados por Suganuma (2008) cinco anos após o plantio das mudas no reflorestamento e um ano antes da montagem do experimento de remoção de sementes.

Study sites	Native Forest	Restoration site
Litter mass ($Mg.ha^{-1}$)	5.1	7.2
Basal area ($m^2.ha^{-1}$)	33.6	24.5
Canopy tree height (m)	~15	~10
Canopy cover (%)	94	84
Grass cover (%)	–	9%

According to Köppen's climatic classification system, the region has a Cfa humid subtropical climate, with hot and humid summers (23.8 ºC average temperature and 194.6 mm average rainfall in January), and a winter with less rain and sporadic frosts (16.8 ºC average temperature and 95.9 mm average rainfall in July). Average annual rainfall is 1,600 mm (Instituto Agronômico do Paraná – IAPAR, 2000; 2011). The soil is a Eutroferric Red Latosol, originated from basaltic rock (Empresa Brasileira de Pesquisa Agropecuária – EMBRAPA, 2011).

2.2 Experimental Design

In the year 2008 (6.5 years after planting), we examined seed removal and seed damage as a surrogate for seed predation in the study sites. Six species were studied, all native to the region: *Strychnos brasiliensis* (Spreng.) Mart. (Loganiaceae), *Ocotea silvestris* Vattimo (Lauraceae), *Poecilanthe parviflora* Benth. (Fabaceae-Papilionoideae), *Copaifera langsdorffii* Desf. (Fabaceae-Caesalpinoideae), *Eugenia florida* DC. (Myrtaceae) and *Cytharexylum myrianthum* Cham. (Verbenaceae). Most species are animal-dispersed (except *P. parviflora*, which is wind-dispersed) and non-pioneer shade-tolerant (except *C. myrianthum*, which is pioneer). Seeds were collected in the region, washed in water and dried in the shade to remove the aril, except for *P. parviflora*, which only required drying.

We used 500 seeds for each species, divided among five plots in the area being restored and five in the native forest (n = 5). Each plot contained 50 seeds, distributed in a 50 x 50 cm area. All plots were placed on flat terrain and delimitated by bamboo stakes. In the forest fragment, the plots were located at 150 m from the forest edge. The seeds were randomly deposited in the soil surface, and mixed with the litter. Seeds were monitored at 15 dayintervals during 60 days. At each monitoring period, both the seeds and the litter were transferred manually to a plastic tray. The bare soil, as well as the material in the tray were screened. Intact and damaged seeds were recorded, and all the material (seeds and litter) was returned to the plot. Seeds not found were considered removed on the plot. Evident damages to the seeds (e.g., parts of seeds in nests, bored and gnawed seeds, presence of pathogens) were recorded.

The non-parametric Mann-Whitney U test ($\alpha = 0.05$) was used for detecting differences in damage and removal of seeds between sites and among species.

3 RESULTS

Seed removal, from the whole set of species, was higher in the native forest (56%) than in the restoration site (16%; Mann-Whitney U statistic = 164.5, $p < 0.001$) and was more frequent during the first 15 days, decreasing after that (Table 2).

A colony of leaf-cutter ants (*Atta* sp.) surged near a plot in the restoration site, but the exclusion of this plot from the analysis did not affect the overall seed removal rate (56.4% in the native forest and 6.4% in the restoration site; Mann-Whitney U statistic = 77, $p < 0.001$). However, such exclusion resulted in near 10% less seeds removed in the restoration site and an increase from two to four species showing significantly higher seed removal in the forest fragment (Table 3).

The percentage of damaged seeds were low and did not differ between sites (3.6% in the forest fragment and 7.6% in the reforestation site) for whole set of species. However, *Ocotea silvestris* and *Copaifera langsdorffii* showed more damage in the reforestation site (4.8% and 5.2%, respectively) than in the native forest (0% and 0%, respectively).

Table 2. Percentage of seeds removed in the first 60 days after disposal in a native forest (NF) and a restoration site (R), northern Paraná state, southern Brazil. Bold numbers indicate differences of seed removal between native forest and restoration site by species (Mann-Whitney U test, α = 0.05).

Tabela 2. Porcentagem de remoção de sementes durante os primeiros 60 dias de exposição em uma floresta nativa (NF) e uma área em restauração (R), norte do estado do Paraná, Sul do Brasil. Números em negrito indicam diferenças na remoção de sementes entre a floresta nativa e a área em restauração por espécie (Mann-Whitney U test, α = 0.05).

Species	15 days			30 days			45 days			60 days		
	NF	R	*p*	NF	R	*p*	NF	R	p	NF	R	*p*
Strychnos brasiliensis	5.6	0.4	0.060	0.8	0.4	0.916	1.2	0	0.060	0.4	3.2	0.754
Ocotea silvestris	**81.2**	**1.2**	**0.009**	14.4	0,0	0.117	**0.8**	**0**	**0.009**	0	0	0.117
Poecilanthe parviflora	63.6	21.2	0.117	11.2	1.6	0.117	4.4	6.8	0.117	3.2	9.2	0.528
Copaifera langsdorffii	**59.6**	**2.4**	**0.016**	3.6	0.4	0.464	**0.4**	**4.8**	**0.016**	3.6	10.8	0.754
Eugenia florida	32.4	12	0.210	9.2	0.8	0.53	2	0	0.09	25.6	3.2	0.250
Cytharexylum myrianthum	7.6	15.2	0.296	0	1.2	0.117	1.2	0.4	0.347	3.2	0.4	0.250

Table 3. Percentage of seeds removed in a native forest (NF) and a restoration site (R) for whole set of seeds after 60 days with all replicates (n = 5) and excluding a plot near leaf-cutter ants (n = 4) in northern Paraná, southern Brazil. Bold numbers indicate differences in seed removal between NF and R by species (Mann-Whitney U test, α = 0.05)

Tabela 3. Porcentagem de sementes removidas em floresta nativa (NF) e um plantio de restauração (R) para o conjunto das sementes após 60 dias (n = 5) e na exclusão da parcela próxima da colônia de formigas (n = 4), norte do estado do Paraná, Sul do Brasil. Números em negrito indicam diferenças na remoção de sementes entre NF e R por espécie (Mann-Whitney U test, α = 0.05).

Species	Removed (n = 5)			Removed (n = 4)			Damages		
	NF (%)	R (%)	*p*	NF (%)	R (%)	*p*	NF (%)	R (%)	*p*
Strychnos brasiliensis	8.4	4.0	0.251	7.5	5.0	0.470	4.8	0.8	0.144
Ocotea silvestris	**96.4**	**1.2**	**0.009**	**95.5**	**1.5**	**0.021**	**0.0**	**4.8**	**0.037**
Poecilanthe parviflora	82.4	38.8	0.144	**80.0**	**23.5**	**0.043**	14.8	34.4	0.251
Copaifera langsdorffii	67.2	18.4	0.060	**68.5**	**1.5**	**0.030**	**0.8**	**5.2**	**0.016**
Eugenia florida	**69.2**	**16.0**	**0.022**	**74.0**	**5.5**	**0.021**	1.2	0.8	0.465
Cytharexylum myrianthum	12.0	17.2	0.403	13.0	3.0	0.194	0.0	0.0	1.000

Four species had more than 65% of their seeds removed in the forest fragment after 60 days: *Ocotea silvestris*, *Poecilanthe parviflora*, *Copaifera langsdorffii* and *Eugenia florida*, showed higher removal rates than *Strychnos brasiliensis* and *Cytharexylum myrianthum*. In the restoration site, the removal rate of seeds of *P. parviflora* was higher than that of *S. brasiliensis* and *O. silvestris* (Figure 2). However, the remaining seeds of *O. silvestris* in the restoration site appeared to be dehydrated and thus non-viable.

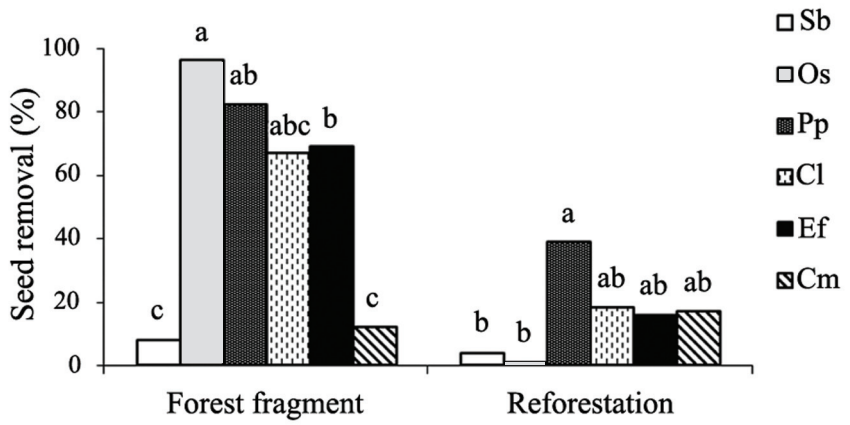

Figure 2. Percentage of seeds of *Strychnos brasiliensis* (Sb), *Ocotea silvestris* (Os), *Poecilanthe parviflora* (Pp), *Copaifera langsdorffii* (Cl), *Eugenia florida* (Ef), and *Cytharexylum myrianthum* (Cm) removed from a restoration site and a native forest, northern Paraná state, southern Brazil. Species followed by the same letter in each treatment do not differ in seed removal rate by site (Mann-Whitney U test, $\alpha = 0.05$).

Figura 2. Porcentagem de sementes de *Strychnos brasiliensis* (Sb), *Ocotea silvestris* (Os), *Poecilanthe parviflora* (Pp), *Copaifera langsdorffii* (Cl), *Eugenia florida* (Ef) e *Cytharexylum myrianthum* (Cm) removidas em uma floresta em restauração e em floresta nativa, norte do Estado do Paraná, Sul do Brasil. As espécies seguidas da mesma letra não diferem entre si em taxa de remoção por área (Mann-Whitney U teste, $\alpha = 0.05$).

4 DISCUSSION

Seed removal rate varied in response to habitat, but our hypothesis was rejected, with higher seed removal in the forest fragment than in the restoration site. In spite of the restoration site presents lower density of regeneration plants, the proximate cause for this shall not be seed predation, and thus the possible presence of disturbance-adapted seed predators in the restoration site, as suggested by Olifiers et al. (2005) findings, did not showed to be important in our study site.

On the other hand, high levels of seed removal in forest interiors than edge sites have previously been reported (Holl and Lulow, 1997; Baldissera and Ganade, 2005; Vélez-Garcia and Pérez-Torres, 2010). These later authors suggests the existence of some open-vegetation avoidance by rodents, which could help to explain our results. Thus, the lower seed removal rate that we observed in the restoration site may be a pattern typical of forest edges and secondary forests, especially during the day and in open canopy sites, indicative of lower activity of small vertebrates in these areas (Nepstad et al., 1996; Cintra, 1997; Vélez-Garcia and Pérez-Torres, 2010).

Other studies have also reported a higher seed removal rate in the first days of exposure. Holl and Lulow (1997) recorded 63% removal during the first 30 days, with 11% occurring in the first 24 hours. Wenny (2000) reported nearly 100% removal of *O. endresiana* seeds in the first 20 days, highlighting the advantages of early germination to escape from seed predation.

While removed seeds may be secondarily dispersed and not always predated (Vander-Wall et al., 2005), our field observations suggest that removed seeds are eaten after removed, similar to the reported by Wenny (2000). Other studies have identified ants as important seed predators in mature and early successional forests (Nepstad et al., 1996; Holl and Lulow, 1997; Garcia-Orth and Martinez-Ramos, 2008) and in our study, leaf-cutting ants are important in damage, fragment and remove seeds in the restoration site, suggesting that the management of leaf-cuting ants in ecological restoration may be a critical issue. For some tree species, however, partial damage is not necessarily lethal to the seed, possibly because such damage occurs without causing harm to the embryo (Vallejo-Marín et al., 2006).

The high density of seeds used in this experiment may have enhanced density-dependent predation. However, even that an experimental design with seeds in lower densities or distributed individually may result in lower removal rates, the density-dependent effect likely was similar in both of our sites. Nonetheless, our findings clearly show that post-dispersal seed removal is very low in restoration sites, suggesting that seed removal/predation is not a barrier to the natural regeneration of tree species in these areas.

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