Mortality and mechanical damage of seedlings in different size fragments of the Brazilian Atlantic Forest

RITA DE CÁSSIA QUIETE PORTELA¹* & FLAVIO ANTONIO MAÊS DOS SANTOS²

¹Post-Graduate Program in Plant Biology, Department of Botany, Institute of Biology, State University of Campinas, CP 6109, Campinas, SP 13083-970, Brazil
²Department of Botany, Institute of Biology, State University of Campinas, CP 6109, Campinas, SP 13083-970, Brazil

Abstract: We assessed the mechanical damage caused by litterfall and vertebrates to seedlings and saplings at the edge and in the interior of three small (14, 20, 29 ha) and one large (9400 ha) fragments of the Atlantic Forest, São Paulo, Brazil. We monitored mechanical damage (e.g. litterfall, vertebrate and human damage) of artificial seedlings, and mortality rates for natural seedlings for 1 year at 3 month intervals. Mechanical damage to artificial seedlings was mainly caused by litterfall (68.1%). Damage to artificial seedlings was approximately two times higher in the smaller fragments than in the large fragment, but the mortality of natural seedlings was not related to fragment size. Mechanical damage and mortality did not differ between edge and interior plots. Although it occurred only occasionally (3.78% of total damage) human impact (trampling) seems to be the most important single cause of seedling mortality.

Resumen: Evaluamos el daño mecánico causado por la hojarasca y los vertebrados a plántulas e individuos juveniles en el borde y el interior de tres fragmentos pequeños (14, 20, 29 ha) y uno grande (9400 ha) de Bosque Atlántico, São Paulo, Brasil. Monitoreamos el daño mecánico (e.g. caída de hojarasca, daño por vertebrados y por humanos) de plántulas artificiales, así como las tasas de mortalidad para plántulas naturales, cada tres meses durante un año. El daño mecánico en las plántulas artificiales fue causado principalmente por la hojarasca (68.1%). El daño en las plántulas artificiales fue aproximadamente dos veces mayor en los fragmentos pequeños que en el grande, pero la mortalidad de plántulas naturales no se relacionó con el tamaño del fragmento. El daño mecánico y la mortalidad no difirieron entre las parcelas del borde y las del interior. Aunque se presentó sólo de forma ocasional, (3.78% del daño total), el impacto humano (pisoteo) parece ser la causa más importante de mortalidad.

Resumo: Acessamos os danos mecânicos causados por serapilheira e vertebrados a plântulas e jovens na borda e interior de três fragmentos pequenos (14, 20 e 29 ha) e um grande (9400 ha) de Mata Atlântica em São Paulo, Brasil. Nós monitoramos danos mecânicos (e.g. serapilheira, vertebrados e danos humanos) em plântulas artificiais e taxa de mortalidade em plântulas naturais por um ano com intervalo de três meses. Dano mecânico em plântulas artificiais foi causado principalmente por serapilheira (68,1%). Dano nas plântulas artificiais foi aproximadamente duas vezes maior nos fragmentos pequenos do que no fragmento grande, mas a mortalidade das plântulas naturais não foi relacionada com o tamanho do fragmento. Dano mecânico e mortalidade não diferiram entre as parcelas da borda e interior. Apesar de ter ocorrido apenas ocasionalmente (3,78% do total de dano), o impacto humano (pisoteio) parece ser a causa mais importante de mortalidade.

* Corresponding Author; e-mail: rita@quiteteportela.com.br
Key words: Artificial seedling models, Atlantic Rain Forest, Brazil, fragmentation, habitat loss, seedling mortality.

Introduction

The process of forest fragmentation results in three distinct changes in the forest ecosystem pattern: reduced forest area, increased isolation of resulting remnants, and the creation of edges where remnant forest abuts modified ecosystems (Kupfer et al. 2006). Each of these changes generates alterations in ecosystem processes such as plant-animal interactions (Aizen & Feinsinger 1994), plant-pathogen interactions (Benítez-Malvido et al. 1999), nutrient cycling (Sizer et al. 2000), and often results in habitat decline and loss of species (Laurance et al. 2002).

The successful regeneration of tree species is influenced by light levels (Dalling et al. 1999), gap size, seed dispersal (Schupp 1988), predation and parasitism during early ontogenetic stages (Cadenasso & Pickett 2000), and physical damage caused by litterfall and vertebrates (Clark & Clark 1989; Ickes et al. 2001; Mack 1998; McCarthy & Facelli 1990; Molofsky & Augspurger 1992). Litterfall and vertebrate trampling are the principal causes of seedling mortality from mechanical damage in tropical forests. The presence of a deep litter layer may also increase seedling mortality, either by increasing their exposure to pathogens or by increasing the damage from digging activities of vertebrates (García-Guzmán & Benítez-Malvido 2003). Accumulated leaf litter also forms a physical barrier to seedling emergence (Santos & Válio 2002). Additionally, a thick litter layer may also affect seedling growth through factors related to light, soil temperature and pH, nutrient and water availability, and even allelopathy (Ahlgren & Ahlgren 1981). The influence of litter depends both on the species that produce it and on the species of seedlings (Ahlgren & Ahlgren 1981).

Physical damage to small plants in tropical forests can be assessed using models of artificial seedlings (Clark & Clark 1989). Such damage varies, according to published reports, between 22% (Scariot 2000, in central Amazonia) and 82% individuals per year (Clark & Clark 1989, in Costa Rica). The wide variation in mechanical damage rates seen in these studies may partly result from methodological differences. Mack (1998), for example, used a more restrictive definition of damage than did Clark & Clark (1989), although both studies used the same artificial models. Other differences may be related to differences between the studied areas such as productivity (Gillman & Ogden 2001), vegetation composition (Gillman et al. 2004; Gillman & Ogden 2005), and the animals present (discussions in: Clark & Clark 1989; Scariot 2000). The degree of habitat disturbance will also influence physical damage to seedlings.

Until recently, little was known about the effects of forest fragmentation on seedlings (see Scariot 2000; Sizer & Tanner 1999); one of the most crucial and vulnerable phase in a plant's life cycle (Scariot 2000). Seedlings, together with seed banks and seed rain, are essential to the recovery of forest vegetation at fragment edges (Benítez-Malvido & Martínez-Ramos 2003a, 2003b). However, seedlings are vulnerable (Mack 1998), they are the first to suffer the effects of fragmentation (Scariot 1999). Fragmentation tends to change the species composition of seedlings (Benítez-Malvido & Martínez-Ramos 2003a), and edge effects influence seedling species recruitment in forest fragments (Benítez-Malvido & Martínez-Ramos 2003b).

The Brazilian Atlantic Rain Forest contains one of the highest levels of species diversity in the world (Fonseca 1985). This forest originally covered an area ca 1.1 million km², corresponding to 12 percent of the land surface of the country, and stretching for >3300 km along the eastern Brazilian coast between the latitudes of 6 and 30ºS (SOS Mata Atlântica 2008). However, a long history of land clearing and the concentration of the largest part of the country's population nowadays make it one of the most-threatened tropical forest ecosystems in the world (Silva & Tabarelli 2001). It is reduced to only ca 7.5% of its original cover (SOS Mata Atlântica 2008) and most remnants consist of small (less than 100 ha), privately owned fragments (Ranta et al. 1998).
These fragments are frequently exposed to human disturbance such as hunting, logging, and plant harvesting, further exacerbating fragmentation effects (Tabarelli et al. 2004).

We compared levels of mechanical damage on artificial seedlings and mortality rates of natural seedlings within fragments of different size. We addressed the following questions: (1) Do rates of mechanical damage to models and mortality to natural seedlings vary according to fragment size? (2) Do these rates change with distance from the fragment edge? Answering these two questions will aid in the evaluation of studies performed with artificial seedlings, and will also be relevant for conservation efforts in the Atlantic Forest because of the key role of seedling establishment in forest regeneration.

**Materials and methods**

**Study site**

Our Brazilian Atlantic Forest study sites were in Caucaia do Alto, located in the Cotia and Ibiúna municipalities, State of São Paulo, where < 14 percent of the original Atlantic Forest of the state remains (SOS Mata Atlântica 2008). We worked in one large and three smaller forest fragments. The large forest is the Morro Grande Reserve, which comprises 9400 ha of secondary and mature forest (old-growth forest). It is located on the border of the São Paulo Atlantic Plateau (23°35’S - 23°50’S; 46°45’W - 47°15’W; altitude 898-979 m). This reserve has been protected for approximately 80 years. It is the only large, well-preserved forest area in this region of São Paulo state. The fragmented landscape extends southwestwards from the reserve, and is dominated by open habitats, which cover 58% of the landscape (33% agricultural fields, 15% areas with rural buildings or urban areas, and 10% native vegetation in early stages of regeneration) (Pardini et al. 2005). Secondary forests cover 31% of the landscape, and pine and eucalyptus plantations cover 7% (Pardini et al. 2005). The three smaller study areas (14, 20, and 29 ha) are located in this fragmented landscape. They are isolated, irregularly shaped, and comprise secondary forest from 50 to 80 years old at altitudes of 904-1003 m. They are located approximately 14 km from the Morro Grande Reserve. Both the Reserve and the rural landscape have similar geologic, geomorphologic, climatic, and vegetational conditions, which make them suitable for comparison (Uezu et al. 2005).

The mean maximum yearly temperature is 27 ºC and mean minimum temperature is 11ºC in this region (SABESP 1997). Rainfall is around 1300-1400 mm year⁻¹ and varies seasonally, with the driest and coldest month between April and August (SABESP 1997). The vegetation in the region is a transition between the coastal Atlantic rain forest and the Atlantic semi-deciduous forest, being classified as “Lower Montane Atlantic Rain Forest” (Oliveira-Filho & Fontes 2000).

**Data collection**

Within each of the three smaller forest fragments, 10 x 100 m plots were delimited at both 5 m (edge plot) and 100 m (interior plot) from, and parallel to, the forest edge. In the large forest area, three pairs of edge and interior plots were delimited. The plot pairs within this largest area are referred to herein as plots 1, 2, and 3 (edge and interior). The edge plots were also delimited at 5 m distant from the forest edge. Within this large forest area, the distance between the edge and interior plots were 4.3, 3.9 and 4.1 km respectively. The mean distance between the edge plots themselves was 2.5 km, and the mean distance between the interior plots was 1.8 km. This large spacing was used to minimize spatial autocorrelation among replicates in the largest area. The 100 m long border of each plot was placed parallel to the forest edge, in order to minimize within-plot effects of the edge-to-interior gradient.

**Mechanical damage and mortality**

Mechanical damage to seedlings was assessed using artificial seedling models, as first used by Clark & Clark (1989). These artificial seedlings are insensitive to environmental stress (drought, shade, etc.) and to biotic agents (competition, predators, etc.), and thus allow the investigator to quantify only direct physical damage to seedlings.

Unlike the original study with artificial seedlings, which used white plastic drinking straws (Clark & Clark 1989), in this study the artificial seedlings were made of two green plastic straws (to avoid attracting visually oriented animals), stapled to form a cross, and with a 10 cm wire “root” fixed at the base. Also unlike from the original study, which used seedlings 20 cm tall and with a 20 cm crosspiece representing the leaves,
here they were 10 cm high, with a 6 cm crosspiece representing the leaves. This size represents the most common above-ground dimensions of 600 naturally occurring seedlings (defined as plants <1 m tall) at the study sites (Portela & Santos 2003).

Each of the twelve 10 x 100 m plots was subdivided into 1 m² subplots. Lots were drawn to randomly select five subplots (= experimental station), each of which received 20 artificial seedlings. The density of naturally occurring seedlings in the Morro Grande Reserve forest ranges between 1 and 53 individuals m⁻² (Portela 2002). Thus a total of 1200 plastic-straw seedlings (100 per plot) were installed (September 2000) and followed for 1 year. Additionally, in each 1 m² subplot the 20 naturally occurring seedlings nearest to the 20 model seedlings that were less than 1 m tall were also marked, to compare their mortality rates with the rates of mechanical damage to the artificial seedlings. The experimental stations were monitored at 3 month intervals for one year (December 2000, and March, June, and September 2001).

Seedling models were classified as damaged when they showed an inclination of 45º or greater. The mode of damage to the artificial seedlings was classified into five categories: (1) caused by litterfall, when a seedling was covered with litter; (2) caused by vertebrates, when a seedling was trampled; (3) unknown cause, when it was impossible to determine what caused the damage but the individuals were still present; (4) missing; and (5) caused by human actions, when footpaths were found or trees had been cut down at the experimental station and as a consequence the seedlings were damaged. Natural seedlings were classified as living, missing, or dead, because of the difficulty in identifying the nature of damage.

Damaged artificial seedlings were removed from the experiment after each assessment, and replaced by new ones, in order to estimate the probability of each kind of damage occurring during the sampling intervals without considering density modifications. By the same reasoning, dead or missing natural seedlings in each sampling were replaced. If a natural seedling died, a nearby natural occurring seedling replaced the dead one; and if a model disappeared, a new one was planted.

**Data analysis**

Percentage of total damaged seedlings, litter damaged seedlings and dead seedlings were analyzed with a Repeated Measures ANOVA, with fragments and edge plots x interior plots as factors and with time dependent measures (von Ende 1993), performed with Statistica 6.0 software (Statsoft 2001).

The D’Agostino-Pearson test for normality for \( n \geq 20 \) was performed using BioEstat software (Ayres et al. 2000). Because the damage caused by litter and the natural seedling mortality were expressed as a percentage, these variables were normalized by arcsine-square-root transformation to conform to the requirements of ANOVA (Zar 1999).

**Results**

Mechanical damage to artificial seedlings was mainly caused by litterfall (68.1%), and appeared to be the main factor influencing natural seedling...
survival (Table 1). The rates of total damage to artificial seedlings varied significantly among the fragments studied (p<0.001, Table 1), and were two times higher in the smaller fragments (22.3%) than in the large fragment (11.2%). Total damage did not differ between edge and interior plots (p=0.180, Table 1). Damage rates differed between the seasons (p=0.005, collection time, Table 1). The higher damage was in the last collection because of the damage caused by (non-human) vertebrates and by human actions. Other causes were not significant.

The analysis of litterfall damage represents almost the same as the total damage analysis because the majority of damage was caused by litter. The rates of damage caused only by litterfall to artificial seedlings varied significantly among the fragments studied (p=0.002, Table 1), and were approximately two times higher in the smaller fragments than in the large fragment (Fig. 1).

![Fig. 1. A and B – Damage by litter (maximum number of individuals of artificial seedlings, = 20 per subplot) C and D – mortality (maximum number of individuals of naturally occurring seedlings, = 20 per subplot) in the six study areas and at the edge and in the interior. In the box plot, the center horizontal line marks the median of the sample. The length of each box shows the range within which the central 50% of the values fall, with the box edges at the first and third quartiles. Values between the inner and outer hinge are plotted with asterisks (*). Outliers are plotted with empty circles (º).](image-url)
Litterfall damage did not differ between edge and interior plots (p = 0.091, Fig. 1). Damage rates did not differ between the seasons (p = 0.107, collection time), and other factor interactions were not significant.

Damage caused by (non-human) vertebrates was infrequent (1.13% of total damage) in the areas studied, occurring at only two stations throughout the study (both in a small forest fragment of 14 ha). One occurred in the interior (September 2001), when five artificial seedlings were damaged, three natural seedlings died, and one was missing. The second occurred at the edge, where four artificial seedlings were damaged.

Unidentified damage to the artificial seedlings represented 19.5% of total damage, and occurred in all fragments. A total of 34 artificial seedlings disappeared from 21 stations, 4.2% of total damage. The largest number of missing seedlings at any single station was 13 (65% of the seedlings), and was due to human intervention.

The damage caused by human actions occurred at low frequency (3.78% of total damage); only three events during the entire study period, although they were intense. The first event occurred in the interior of one smaller fragment (March 2001) and is described was the statement above, when five artificial seedlings were damaged. The second event occurred close to the edge of another smaller fragment (June 2001), when 10 artificial seedlings were damaged and one was found missing. The third event occurred close to the edge of the largest fragment (September 2001), where 15 seedlings were damaged and one was noted as missing. The first event was due to logging, and the other two to footpaths opened in the forest. Damage caused by human activities directly affected seedling mortality; the station with 15 damaged artificial seedlings was also the station with the highest seedling mortality rate encountered in this study (7 individuals). The station with five damaged artificial seedlings had the second-highest natural seedling mortality rate (5 individuals), as well as the highest occurrence of missing seedlings (11 individuals). Five other seedlings were also found dead in the edge plot of the 14 ha fragment (June 2001). Human activities are unpredictable and can occur at any time of the year.

Mortality of natural seedlings did not vary among the fragments studied (p = 0.686, Table 1), between edge and interior plots (p = 0.612, Table 1), or among the seasons of the year (p = 0.614) (Fig. 1, Table 1). But the difference between edge and interior plots was significant in the last collection, where the edge showed higher mortality than the interior plots due to cleaning of a foot path, or logging.

**Discussion**

Our results suggest that smaller fragments had greater litterfall and total damage to artificial seedlings, but mortality of natural seedlings did not show a response to fragment size, perhaps because mechanically damaged seedlings are able to resprout, while artificial seedlings do not. Mortality of natural seedling was greatest due to stochastic events, which in our case were human causes, like logging and clearing.

Because artificial seedlings tend to suffer more total and especially litterfall mechanical damage in smaller forest fragments, this factor may present a major barrier to the establishment and regeneration of forest vegetation (Ahlgren & Ahlgren 1981; García-Guzmán & Benítez-Malvido 2003; Santos & Válio 2002). This result contrasts with that of Scariot (2000), who found similar “mortality rates” among artificial seedlings due to litterfall after 1 year for 1, 10, 100 ha fragments, and for continuous forest in the Amazon. The differences between these two studies may be related to historic differences of the regions, especially the kind of forest (Atlantic and Amazon forest), age (50 to 80 years old and 20-30 years old) and the size of the fragments.

The occurrence of total and litterfall damage did not seem to be directly linked to seedling mortality, because the site with the highest number of damaged seedlings did not have the highest mortality rate. Similar results were found by Gillman et al. (2002) in New Zealand. This lack of correlation may be related to the survival strategies of natural seedlings (Gillman & Ogden 2001, 2005) and their capacity to resprout (Gillman et al. 2002). Nonetheless, the use of seedling models permit the creation of a damage index (independent of time, species, and place) with which to compare different systems. According to Gillman et al. (2002), seedling models can aid in quantifying the potential damage resulting from litterfall, but not mortality, because they tend to overestimate the latter. The same was found in this study.

Damage caused by vertebrates (other than humans) did not seem to be important in terms of seedling dynamics in the study area. Such damage
only occurred once, in a fragment where squirrels (*Sciurus aestuans*) had buried fruits of *Syagrus romanzoffiana*. Both palm trees and squirrels were common in this fragment (personal observation). Scariot (2000) also found that damage attributable to vertebrates was not important to seedling dynamics in Amazonian forest fragments. In this study, damage by animals to artificial seedlings does not represent an estimate of animal damage to true plants in the wild, and was not analyzed further. Other studies have found that vertebrates significantly influence seedling mortality (de Steven & Putz 1984; Ickes et al. 2001). Although it occurred only occasionally (three events during the entire study period), damage caused by human activities was the single most harmful factor (75% of artificial seedlings damaged at one experimental station), and caused the highest mortality (35% of natural seedling mortality at one experimental station). This indicates that human activities may significantly affect seedling survival and dynamics in this forest, especially near roads, towards certain tree types, along game routes, etc. Some studies have already reported the influence of human activities on the recruitment of tree species (see Forget et al. 2001). Forget et al. (2001) found low seed-seedling survival of *Carapa procera* (Meliaceae) in a logged forest in French Guiana and it may have been a result of a decline in mammalian seed predators and dispersers in the logged forest due to hunting.

Seedling mortality does not appear to be related directly to forest fragment size. This might be expected because of the large environmental heterogeneity commonly observed in tropical forests (Martínez-Ramos et al. 1988; Scariot 2000; Svenning 2001). Santos & Válio (2002) observed wide temporal and spatial variation in seedling recruitment in a south-eastern Brazilian semi-deciduous forest. Martínez-Ramos et al. (1988) confirmed significant spatial heterogeneity in a tropical forest in Mexico, largely caused by perturbations such as tree-falls. This heterogeneity was observed at scales as small as 25 m², which implies that neighbouring 5 × 5 m areas in this forest can show different perturbation frequencies. Accordingly, the effects of fragment size may have much less influence on seedling mortality than the dynamics related to tree falls and gap formation (Martínez-Ramos et al. 1988).

Our data on seedling mortality did not indicate any influence of edge-effect or collection time. According to Meiners et al. (2002), edge-effects on seedling recruitment depend on the species under consideration. Because the present study analysed an entire community, and not a particular species, patterns may be harder to detect. In contrast, Benítez-Malvido (2001), working in the Amazon with three native tree species of Sapotaceae, found that mortality rates were higher in smaller fragments, but that edge effects were more important in larger fragments. Again, like the mechanical damage, the differences between these two studies may be related to historic differences of the regions, especially the age and the size of the fragments.

Finally, parameters not examined here, such as soil type, wind exposure, and the history of past perturbation at each site may have influenced the results. The impact of each microhabitat on seedling mortality would imply wide spatial variation in the probability of seedling establishment. Forests can be considered dynamic mosaics of different ages produced by perturbations, and influenced by different biotic and abiotic conditions (Martínez-Ramos et al. 1988; Tabanez & Viana 2000). The single most important factor clearly and directly influencing seedling mortality in the present study was the impact caused by footpaths within the forest and by logging. This observation demands consideration, because most of the remaining Brazilian Atlantic Coastal Forest is not protected from ongoing human impact. On the other hand, there was a clear fragmentation effect on artificial seedling damage. While damage in this study was not related to mortality, multiple occurrence of damage to the same seedling (or perhaps even single occurrences) would likely increase mortality potential in the future.

Acknowledgements

We thank L.F. Alves, B. Canela and R. Belinello for help in the fieldwork; and D. A. Clark, W. F. Laurance, L. F. Alves, M. G. Fonseca and F. N. Ramos for useful suggestions on an early version of the manuscript. Financial support was provided by FAPESP (Proc. No. 99/05123-4, 00/00596-0). F.A.M. Santos was supported by a grant from the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, Grant No. 207132/2004-8). This study is part of the project “Biodiversity Conservation in fragmented
landscapes on the Atlantic Plateau of São Paulo – BIOTA/Caucaia project."

References


