Seed production, pre-dispersal seed predation and germination of *Nothofagus glauca* (Nothofagaceae) in a temperate fragmented forest in Chile

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Abstract

The ecological processes and interactions that involve plants at the seed level may be altered in a fragmented forest, with consequences on plant recruitment and forest structure. We studied seed production, pre-dispersal seed predation and germination of non-damaged and damaged seeds of the endemic and vulnerable *Nothofagus glauca* tree, in small fragments and in a continuous area of the Maulino forest, in the Mediterranean coast of Central Chile. Pre-dispersal seed predation was evaluated in seeds produced throughout two consecutive seasons (2005–2006), while germination was evaluated in the field in the year 2005. During 2006, seed production was 16 times lower in the small fragments and 2.6 times lower in the continuous forest than in 2005. In 2005 there was a similar seed production in continuous forest and small fragments, while in 2006 seed production was significantly lower in small fragments. The microlepidopteran larvae, *Perzelia* sp., was confirmed as a pre-dispersal seed predator of *N. glauca*. Pre-dispersal seed predation occurred in the 57% of *N. glauca* seeds, and it was similar in both the continuous forest and fragments. Germination was low ($<3\%$), and did not vary among small fragments and continuous forest. As expected, germination of non-damaged seeds was significantly higher than preyed upon seeds; nevertheless there were some damaged seeds that germinated. There was no relationship among seed production or intensity of pre-dispersal seed predation with the diameter at breast height, height of tree and the distance to the nearest flowering conspecific. Similarly, there was no relationship among seed germination and canopy openness or soil humidity. Our results, along with those from previous studies, suggest that *N. glauca* populations might be depressed in small remnants of the Maulino forest, because of a decreased seed production and an increased post-dispersal seed predation.

Keywords: Maulino forest; Forest fragmentation; Seed production; Pre-dispersal seed predation; Germination; Seed fate

1. Introduction

The initial stages of the plant life cycle are critical for plant recruitment (Harper, 1977). Among them, seeds correspond to the first stage that interacts with the abiotic and biotic environment, and these interactions will strongly affect the future of plant populations (Harper, 1977). Seed production may be affected by microclimatic conditions, and also by pollination, depending on the quantity or quality of the transferred pollen (Aizen and Feinsinger, 1994; Ågren, 1996; Cunningham, 2000). Seed germination and seedling emergency may also be directly affected by microclimatic conditions and indirectly by pre and post-dispersal seed predation via a reduction in the number of seeds available for germination (Janzen, 1970; Harper, 1977; Powell and Powell, 1987; Schelin et al., 2004; Vallejo-Marín et al., 2006).

The abiotic and biotic conditions where a seed develop are modulated by human disturbances, like habitat fragmentation. This phenomenon consists in the breaking apart of a continuous habitat into a number of smaller fragments of smaller total area, isolated by each other by a matrix of habitats unlike the original (Zabel and Tscharntke, 1998; Fahrig, 2003). In native forest this is a frequent and increasing phenomenon. The smaller forest fragments undergo microclimatic, structural and functional

changes (Saunders et al., 1991) that may result in an altered seed production and seed germination compared with an unfragmented forest (Bruna, 1999; Cunningham, 2000; Ghazoul and McLeish, 2001; Cascante et al., 2002).

The effects of forest fragmentation on seed production, germination, as well as post-dispersal seed predation, have been well studied (Ågren, 1996; Cunningham, 2000; Tomimatsu and Ohara, 2002; Cascante et al., 2002; Donaldson et al., 2002; Brys et al., 2004; Donoso et al., 2004; Henríquez, 2004). However, little it is known about the effects of forest fragmentation on pre-dispersal seed predation. Only two studies have addressed the effects of habitat fragmentation on pre-dispersal seed predation (Cascante et al., 2002; Chacoff et al., 2004). They suggest that the intensity of this interaction is depressed in small fragments, particularly near their edges, probably because of a depression in the abundance, diversity and species richness of seed predators, which are mainly specialised insects that respond to the environmental fluctuations resulting from fragmentation (Didham et al., 1996; Baguette et al., 2003; Chacoff et al., 2004). Both of these works were carried out in tropical and subtropical forests, so it was unknown until now, how pre-dispersal seed predation varies in fragmented temperate forests.

Although the preyed upon seeds have little chance to survive, and thus they do not germinate, pre-dispersal seed predation not always results in death. The lethality depends on the seeds size, the kind of predator and the intensity of the damage to the embryo (Dalling et al., 1997; Vallejo-Marín et al., 2006). Large seeds attacked by insect larvae may suffer only partial damage, with producing a total injury to the embryo. In such a case, seed predation will not reduce germination (Vallejo-Marín et al., 2006). In fact, seed germination may be enhanced by pre-dispersal seed predation, because the orifice originated by the predator in the seed may increase embryo access to soil humidity (Grubb, 1977; Baskin and Baskin, 1998). Such abiotics variables, that are necessary for germination, are also modified by forest fragmentation (Bruna, 1999).

In central Chile, the Maulino forest has been intensively fragmented, affecting the abundance of several plant species such as *Nothofagus glauca* (Phil.) Krasser (Nothofagaceae), an endemic, vulnerable dominant tree (IUCN, 2006). Simulations of the successional dynamic of this forest predict that abundance of *N. glauca* will diminish notably in the small forest fragments, increasing the dominance of sclerophyllous species (Bustamante et al., 2005). This reduction of the relative abundance of *N. glauca* may be the consequence, to some extent, of alterations in the processes and interactions that involve their seeds. It is known that in fragments the intensity of post-dispersal predation of seeds of *N. glauca* increases, as well as their predator abundance (Donoso et al., 2004). Also, the recruitment of seedlings decreases at a higher extent in forest fragments relative to continuous forest (Castillo, 2005). Nevertheless, how pre-dispersal seed predation and germination of seeds of *N. glauca* changes with forest fragmentation was, until now, unknown.

In other few studies there has been reported a decrease in pre-dispersal seed predation with forest fragmentation. But considering that the recruitment of *N. glauca* is diminishing in the small fragments of the Maulino forest, and because seed production, pre-dispersal seed predation and germination are important for the recruitment of new plants (Harper, 1977), we expected a lower seed production, a higher pre-dispersal seed predation and a lower germination in small fragments than in the continuous forest.

2. Material and methods

2.1. Study area

The Maulino forest is a unique temperate ecosystem located in Central Chile, between 35°55′S and 37°20′S and it is one of the 25 global biodiversity hotspots (Myers et al., 2000). This forest harbors several endemic species such as *Gomortega keule* (Gomortegaceae), *Pitavia punctata* (Rutaceae), *Nothofagus pumilio* and *Nothofagus alessandri* (Nothofagaceae), some of them with conservation problems (San Martín and Donoso, 1996). Precipitation is concentrated during winter (116.2 mm) and spring (90.5 mm) (Di Castri and Hajek, 1976). In the last decades this forest has been intensively deforested and fragmented, and nowadays it persists as a mosaic of small fragments surrounded by a matrix of introduced pine plantations (*Pinus radiata*) (Pinaceae) (San Martín and Donoso, 1996; Grez et al., 1997; Bustamante and Castor, 1998; Echeverría et al., 2006).

2.2. Study species

N. glauca is a representative species of the Maulino forest (Donoso and Ramírez, 1994), distributed from 34°01′S to 37°27′S (Le-Quesne and Sandoval, 2001). It is a monoecious and deciduous tree; female flowers produce fruits with three nuts, each with one seed. Seeds are about 18 mm long and 526 mg weight (Rodríguez et al., 1983; Donoso et al., 2004). They mature from November to April, period when seed rain occurs (Donoso and Ramírez, 1994; J.A. Simonetti, unpublished data). The microlepidopteran larva (*Perzelia* sp.) is a pre-dispersal seed predator of *N. glauca*. This insect has an annual life cycle, and its larvae emerge from August to November, similar to the flowering of *N. glauca* (Morales, 1993; Rojas, 1996). Post-dispersal seed predators are small mammals (Donoso et al., 2004). Germination occurs from late July to September, its seeds are desiccation sensitive and do not form a seed bank (Donoso, 1975).

2.3. Experimental design

Observations and experiments were conducted in one area of continuous forest and four small forest fragments. The continuous forest corresponds to the protected area "Reserva Nacional Los Queules" (35°59′S–72°41′W) which along with private neighbours lands cover and area of 600 ha, one of the largest continuous remnant of Maulino forest. Forest fragments vary from 2 to 5 ha, and are separated from each other by at least 50 m (see Donoso et al., 2004 for a map of the area). The

continuous forest and forest fragments are dominated by *N. glauca* trees.

2.4. Seed production

Seed production of *N. glauca* was evaluated monthly throughout two periods: from November to April 2005 and from November to May 2006 (late spring to autumn), covering the whole period of *N. glauca* seed production at the Maulino forest (J.A. Simonetti, unpublished data). Five trees were selected randomly in each forest fragment ($n = 20$) and 20 trees in the continuous forest, 40 trees in total. As *N. glauca* seeds disperse by gravity, seed production was evaluated as the number of seeds collected in two traps of $0.25 \ m^2$, located below the canopy of each tree. Each trap consisted in beds made of synthetic mesh ($1 \ mm^2$ of grid), held up by plastic tubes of 1 m above soil level (Harms et al., 2000). The seeds collected in the two traps under a tree were pooled for the analysis. We also evaluated some variables that could be related to seed production: tree height, diameter at breast height (DBH), distance to the nearest flowering conspecific. Tree height was estimated using trigonometric calculus, considering the distance (meters) from the observer to the tree and determining the angle from the observer to the top of the canopy of each tree with the help of a clinometer (Kohyama et al., 2003). The distance to the nearest flowering conspecific was evaluated as the distance (meters) between the most external branch from each experimental tree and from each nearest flowering conspecific.

2.5. Pre-dispersal seed predation

When falling from the tree, seeds that have been previously preyed upon present a hole through which the larva of the predatory insect has emerged. After falling from the trees, seed coat is very hard, making oviposition by *Perzelia* sp. very unlikely. Moreover, when seed fall from the trees, adults are no longer flying around, so we can exclude infestation of seeds after dispersal (Cruz, 1981). Therefore, the intensity of pre-dispersal seed predation was evaluated as the percentage of perforated seeds over the total seeds collected in the traps.

2.6. Seed germination

Germination was evaluated through a field experiment, using all seeds collected in the first year of the study ($n = 212$ in the continuous forest and $n = 712$ in the forest fragments). The seeds were immediately marked and sowed in 10 cm diameter plastic pots, perforated at the base and located under the same tree where seeds were collected. Each month, two pots of each tree were sown, one with non-damaged and another with damaged seeds, a maximum of ten seeds per pot. The pots were buried at soil level, filled with substrate and covered with leaf litter collected near each experimental tree to mimic natural conditions of the ground. They were excluded from granivorous birds and small mammals using a cone of wire mesh of 0.5 m diameter. Monthly, we sowed 80 pots, 400 along the whole

study period, from December 2004 to April 2005. The percentage of germinated seeds was registered monthly, from July to December 2005, period in which *N. glauca* seeds usually germinate at the Maulino forest (Guerrero, 2004). Seeds were considered germinated when the radicle emerged at least 2 mm from the seed cover (Wenny, 2000). All germinated seeds were marked with threads of wire of different colors to avoid counting again this germination event. The percentage of germinated seeds was obtained dividing the number of germinated seeds over the total number of sowed seeds $\times$ 100. Additionally, possible relationships between the environmental conditions of the micro site (e.g., light and soil humidity) and seed germination were evaluated. Soil humidity was measured in $200 \ cm^3$ of soil samples collected 1 m away from each germination exclusion, through the gravimetric method (i.e., weight difference between wet and dry soil). Soil humidity was evaluated from January to December 2005. The light level was estimated from March to December 2005 through canopy openness, defined as the proportion of clear sky observed from a given point under the tree. This estimation was made using digital pictures taken in every single exclusion, using a Canon Powershot G2, with a maximum focus aperture of 35 mm lens to 53° approximately (Bunnell and Vales, 1990; Jennings et al., 1999). The percentage of lightness was estimated through photographic analyses using the Scion Image 4.02 software for Windows.

2.7. Data analysis

As some samples were taken from the same experimental unit (i.e., 20 samples coming from only one continuous forest, because there is no other large remnant at the Maulino forest), data may have been not statistically independent and may not be used as replicates in the statistical analysis. In order to test for the independence of the samples, and use our data properly in the statistical analysis, we run the Mantel tests (Fortin and Gurevitch, 1993). This analysis showed that the difference in seed production, seed predation and germination between trees or sampling points within the continuous forest and fragments were spatially independent (Mantel test, 10,000 iterations; Table 1). Therefore, each tree or sampling point was considered a replicate.

Table 1
Mantel test to assess the spatial independence in seed production, seed predation and seed germination between sampling points (trees) within fragments and within continuous forest, in 2005 and 2006

Comparison	Forest fragments (r_p, P)	Continuous forest (r_p, P)
Seed production		
2005	0.123, 0.097	0.062, 0.404
2006	−0.021, 0.770	−0.006, 0.988
Seed predation		
2005	0.029, 0.700	0.019, 0.783
2006	−0.080, 0.920	0.062, 0.404
Germination		
2006	−0.048, 0.526	0.120, 0.115

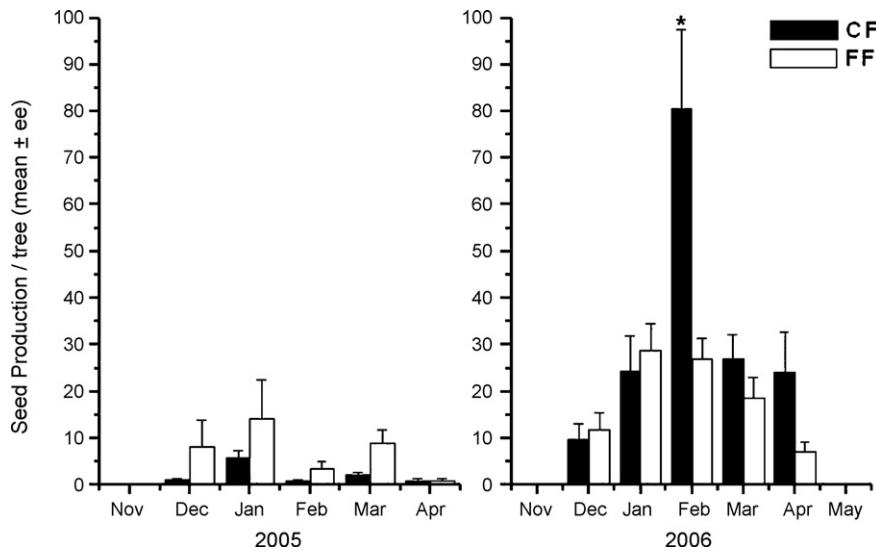Data are Pearson correlations (r_p) and probabilities (P) ($\alpha = 0.05$).

Fig. 1. *N. glauca* seed production/tree during 2005 and 2006 in continuous forest (CF) and forest fragments (FF) (mean ± S.E.). The asterisk on the bar indicates significant differences (Tukey < 0.05).

The difference in seed production and pre-dispersal seed predation between small fragments and the continuous forest was evaluated through an Analysis of variance (ANOVA), with time (months) as a repeated measure and habitat (continuous forest and forest fragments) and year (2005–2006) as fixed factors. The percentage of preyed upon seeds was transformed to arcsine of the square root (Sokal and Rohlf, 1995). Multiple comparisons among months and years were carried out with Tukey test.

The percentage of germinated seeds was evaluated as the quantity of germinated seeds, either non-damaged or damaged, over the total seeds × 100. Germination among habitats was compared through a proportion test (test Z). Germination of damaged and non-damaged seeds was compared using a Logistic Regression Model, assigning 0 to seeds that did not germinate and 1 to those that did germinate.
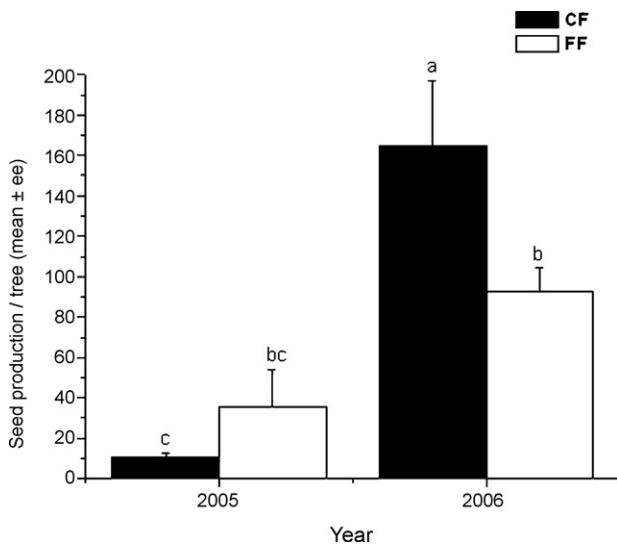
Additionally, regressions analyses were conducted relating seed production and pre-dispersal seed predation with (1) height, (2) DBH and (3) distance to the nearest flowering conspecific of each experimental tree and among seed germination with (1) soil humidity and (2) canopy openness.

3. Results

3.1. Seed production

Both in the continuous forest and in forest fragments, and in both years (2005–2006), *N. glauca* seed production started in December and finished in April (Fig. 1). Cumulative seed production during 2005 was 5.5 times lower than in 2006 (924 seeds versus 5154 seeds), with 2005 seed production 16.5 and 2.6 times lower than in 2006, in the continuous forest and in forest fragments, respectively (Fig. 2). Seed production was higher in the continuous forest than in the small fragments, but this difference was significant only in February 2006, when in the continuous forest there was a three times higher seed production than in the forest fragments (Fig. 1 and Table 2). Cumulative seed production per tree in 2005 was slightly

Fig. 2. Cumulative *N. glauca* seed production/tree during 2005 and 2006 in a continuous forest (CF) and forest fragments (FF) (mean ± S.E.). Means with the same letter did not differ significantly (*P* > 0.05).

Table 2
Results of the rm ANOVA for forest fragmentation effect (habitat), year and month on *N. glauca* seed production

Variation source	d.f.	MS	*F*	*P*
Habitat	1	2199.61	1.46	0.230
Year	1	44732.25	29.69	<0.001
Habitat × year	1	9389.61	6.23	0.014
Error	76	1506.29		
Month	4	5367.23	11.17	<0.001
Month × habitat	4	3183.82	6.62	<0.001
Month × year	4	5879.52	12.23	<0.001
Month × habitat × year	4	2148.07	4.47	0.001
Error	304	480.44		

superior in the forest fragment that in the continuous forest, but this difference was not statistically significant, while during 2006 it was significantly higher in the continuous forest than in small fragments (Fig. 2 and Table 2). The tree height was 17.6 ± 1.3 m, the DBH was 1.64 ± 0.1 m and the distance to the nearest flowering conspecific was $10.5 + 1.02$ m (average + 1S.E. in all cases). None of these variables were significantly correlated with seed production (height of tree: $r^2 = -0.027$, $P = 0.924$, DBH: $r^2 = -0.025$, $P = 0.790$ and the distance to the nearest flowering conspecific: $r^2 = 0.010$, $P = 0.242$).

3.2. Pre-dispersal seed predation

Seeds were preyed upon along the whole production period (December–April), but in both years a higher proportion of seeds were preyed upon at the beginning of the season (Fig. 3 and Table 3). When considering all seeds produced in each season (cumulative seed production), independently of the habitat, the percentage of preyed upon seeds was significantly higher in 2005 than 2006 (65% out of 924 seeds and 51% out of 5154 seeds, respectively; Table 3). In general, there was no significant statistical difference in the percentage of damaged seeds in small fragments and continuous forest (Table 3). During 2005, in average 67% of seeds per tree was preyed upon in the continuous forest (in total 176 out of 212 seeds) and 63% in the forest fragments (576 of 712), while during 2006, an average of 55% of the seeds per tree was preyed upon in the continuous forest (in total 1570 out of 3296 seeds) and 45% in the forest fragments (1151 out of 1858 seeds). There was no significant relationship between seed predation and the height of the tree ($r^2 = -0.026$, $P = 0.863$), DBH ($r^2 = -0.020$, $P = 0.636$) or the distance to the nearest flowering conspecific ($r^2 = -0.011$, $P = 0.458$).

Table 3
The results of the rm ANOVA on fragmentation effect (habitat), year and month on *N. glauca* seed predation

Variation source	d.f.	MS	F	P
Habitat	1	48.9	0.02	0.877
Year	1	21010.7	10.25	0.002
Habitat × year	1	44.8	0.02	0.882
Error	76	2049.2		
Month	4	24715.0	29.26	<0.001
Month × habitat	4	2060.5	2.43	0.047
Month × year	4	2486.3	2.94	0.020
Month × habitat × year	4	745.9	0.88	0.474
Error	304	844.5		

3.3. Seed germination

The percentage of seeds that germinated was very low (2.17%, 20 seeds from a total of 924). A similar percentage of seeds germinated in the continuous forest and in the forest fragments (1.41 and 2.38%, respectively; test of proportions, $Z = 0.60$, $P = 0.54$). Non-damaged seeds germinated significantly more than damaged seeds ($10.39 \pm 3.6\%$ versus $1.3 \pm 1.24\%$; $\chi^2 = 19.44$, $P < 0.001$), and preyed upon seeds only germinated at the forest fragments (three seeds). On the other hand, the canopy openness was significantly higher in the forest fragments than in the continuous forest ($28.5 \pm 2.9\%$ versus $14.7 \pm 2.4\%$) ($F_{(1,38)} = 13.08$; $P < 0.01$). Nevertheless, there was no significant relationship between the canopy openness and the germination of non-damaged seeds ($r^2 = 0.081$, $P = 0.34$) or attacked seeds ($r^2 = -0.027$, $P = 0.980$). The soil humidity was higher in the continuous forest than in the small fragments (27.09 ± 1.02, $20.6 \pm 1.15\%$) ($F_{(1,38)} = 17.47$, $P < 0.01$), but it was not
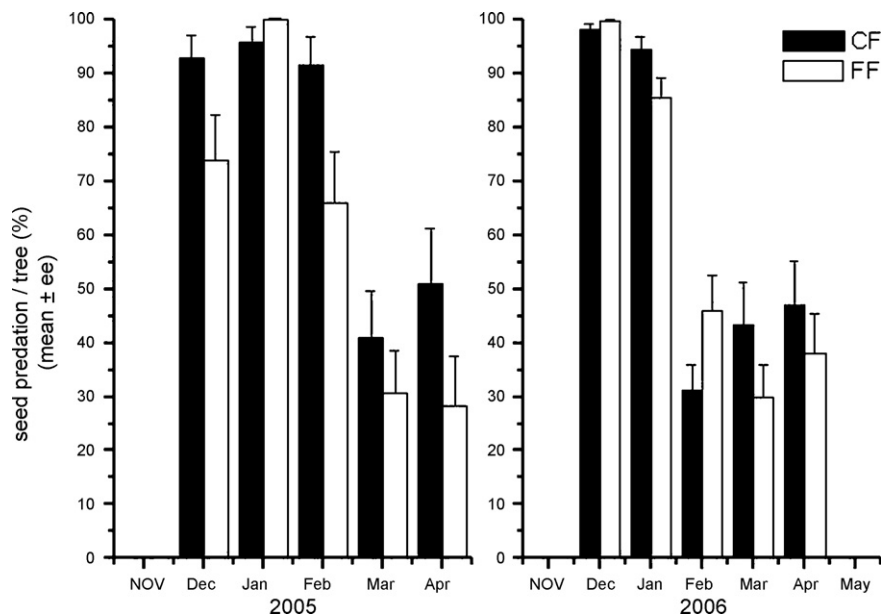

Fig. 3. *N. glauca* seed predation/tree (%) during 2005 and 2006, in continuous forest (CF) and forest fragments (FR) (mean ± S.E.).

related to the germination of non-damaged seeds ($r^2 = -0.027$, $P = 0.689$) or damaged seeds ($r^2 = -0.026$, $P = 0.940$).

4. Discussion

Seed production of *N. glauca* varied strongly among years, being significantly higher in 2006 than in 2005. This annual variability was higher in the continuous forest, with 16 times more seeds produced in 2006 than in 2005, while in the small fragments this increase was only 2.6 times higher. This is not an isolated result since an independent evaluation of the seed rain at the Maulino forest between 2002 and 2005, revealed also that more seeds of *N. glauca* trees are produced every other year, and that this increase in seed production is more evident in the continuous forest than in small fragments (J.A. Simonetti et al., unpublished data). Variation of seed production among years could be due to annual changes of environmental variables that affect this process, for example, in the amount of precipitation (Herrera, 1998), as usually happens in Central Chile (Di Castri and Hajek, 1976). In fact, in the Maulino forest soil humidity was lower in 2004 than in 2005 (J.A. Simonetti, unpublished data), which may have resulted in a lower seed production during the following year (2005).

Furthermore, seed production of *N. glauca* was significantly lower in small fragments than in the continuous forest, but only during 2006, year when in total a higher amount of seeds was produced. During 2005, a slightly higher, but non-significant, seed production occurred in the forest fragments than in the continuous forest, but this occurred because of a single tree that produced a large amount of seeds (52% of the total seed produced in forest fragments). Reduction of seed production in fragmented landscapes is usually associated with a depression in pollination as a result of a reduction of the pollinator fauna (Aizen and Feinsinger, 1994; Murcia, 1996; Jules and Rathcke, 1999; Cunningham, 2000; Aizen and Feinsinger, 2003; Fuchs et al., 2003; Brys et al., 2004; Valdivia et al., 2004). However, *N. glauca* is a wind pollinated species (Donoso, 1975), so factors other than pollination affected seed production. Regressions among the number of seeds produced and DBH, height of tree and distance of each nearest flowering conspecific, indicate that none of these variables would explain the seed production of *N. glauca* neither in continuous forest nor in the forest fragments. The lower seed production in forest fragments may have been also due to pollen limitation as a consequence of a lower density of trees. However, in forest fragments the density of *N. glauca* is approximately twice than in the continuous forest (Bustamante et al., 2005). Therefore, pollen limitation is not a plausible explanation of the lower seed production documented for small fragments. Again, environmental conditions may explain the difference in seed production between forest fragments and continuous forest, since in the continuous forest soil humidity is higher than in the small fragments (Henríquez, 2002).

A high percentage of the seeds produced by *N. glauca* at the Maulino forest (57% of 6078) was preyed previous to dispersal, suggesting that this animal–plant interaction may be an important factor for the regeneration of this species. Contrary to what has been described for both tropical and subtropical forests (Cascante et al., 2002; Chacoff et al., 2004), at the Maulino forest pre-dispersal seed predation of *N. glauca* was not affected by fragmentation, with a similar percentage of attacked seeds in forest fragments and the continuous forest. Because of changes in the environmental conditions in the forest fragments, fragmentation usually modifies the abundance and diversity of insects (Didham et al., 1996), however, this seems not to occur at the Maulino forest, where most herbivorous insects do not change in abundance and number of species with fragmentation (Jaña-Prado and Grez, 2004). Similar to seed production, there was not relationship among the pre-dispersal seed predation and DBH, height and distance to nearest flowering conspecific, which suggest that this plant attributes do not affect this plant–animal interaction.

Capture of insects associated with *N. glauca* canopy, and larvae found in attacked seeds from which adults emerged, confirmed the microlepidoptera *Perzelia* sp. as a predator of *N. glauca* seeds (Morales, 1993; Rojas, 1996). It has been described that microlepidopteran adults fly in spring and their larval stage lasts approximately two months (Morales, 1993; Rojas, 1996). Considering that *N. glauca* seeds fall when larvae have already emerged (A. Burgos, obs. pers.; Rojas, 1996), it is possible to infer that this insects parasite *N. glauca* at the moment of flowering, in early spring.

Germination of *N. glauca* seeds was very low, with only 20 seeds germinating from a total of 924 seeds produced during 2005, the year when germination was evaluated. This low germination was also observed in previous experiments carried out at the Maulino forest with *N. glauca* nursery seeds (with a viability of 95%), from which around 10% germinated (Guerrero, 2004). These small germination values may be related to the fact that seeds of this species are recalcitrant, as in many other native trees in Chile (Donoso, 1975; Bustamante et al., 1996). Because of this life history attribute, seeds should be extremely sensitive to the abiotic conditions prevailing in the soil during the summer at the Maulino forest, being unable to survive and germinate to the next season.

As expected, most seeds that germinated were not preyed previous to dispersal, indicating that pre-dispersal seed predation is important in modulating seed germination. Interestingly, three seeds that were previously attacked by insects germinated and established as seedlings. Nevertheless, this number is extremely low to infer any mutualistic effect of pre-dispersal seed predators on plant recruitment (Baskin and Baskin, 1998).

Although germination may be depressed by fragmentation (Menges, 1991; Bruna, 1999), similar percentages of germination in fragments of varying size have also been described in temperate and tropical zones (Fuchs et al., 2003; Kolb, 2005). *N. glauca* is a shade-intolerant species, so a higher germination might have occurred in forest fragments, where the light conditions would have enhanced germination. Nevertheless, the percentage of seed germinating was similar in the continuous forest and small forest fragments.

In summary, our data showed that seed germination and seed predation did not differ between forest fragments and

continuous forest, then they do not explain regeneration differences between these two habitats. The only process that differed between these two habitats was seed production, but only during one year of study. To what extent differential seed production might explain the higher regeneration documented at the continuous forest relative to forest fragments (Bustamante et al., 2005; Castillo, 2005) will depend on the frequency of years in which seed production becomes higher in continuous forest. Ongoing monitoring of seed production in forest fragments and continuous forest will help us to elucidate the importance of seed production per se to explain regeneration process of *N. glauca*.

5. Conclusion

Fragmentation decreased seed production in only 1 of 2 years, and there was no effect of fragmentation on pre-dispersal seed predation and germination. Thus, there is some (little) evidence for negative effects of forest fragmentation on the reproduction of the species, through ecological changes involving the initial stages of its life cycle, like seed production and post-dispersal seed predation. Pre-dispersal predation and germination would be less relevant as they do not change with forest fragmentation. However, in order to be able to say anything about the future fate of *N. glauca* populations at the Maulino forest, other processes and interactions that occur later in the life cycle should also be considered (Silvertown et al., 1993). This will contribute to the efforts to conserve this vulnerable species of the endangered and unique Maulino forest.

Acknowledgements

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