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Do carbon-based defences reduce foliar damage? Habitat-related effects on tree seedling performance in a temperate rainforest of Chiloé Island, Chile

Abstract Carbon-based secondary compounds (CBSCs), such as phenols or tannins, have been considered as one of the most important and general chemical barriers of woody plants against a diverse array of herbivores. Herbivory has been described as a critical factor affecting the growth and survival of newly established tree seedlings or juveniles then, the presence of secondary metabolites as defences against herbivores should be a primary strategy to reduce foliar damage. We examined whether light-induced changes in leaf phenolic chemistry affected insect herbivory on seedlings of two rainforest tree species, *Drimys winteri* (Winteraceae) and *Gevuina avellana* (Proteaceae). Seedlings of both species were planted under closed canopy and in a canopy gap within a large remnant forest patch. Half of the seedlings in each habitat were disinfected with a wide-spectrum systemic insecticide and the other half were used as controls. Seedling growth, survival, and foliar damage (estimated by an herbivory index) due to insect herbivores were monitored over a period of 16 months (December 2001–April 2003). The total leaf content of phenols and condensed tannins were assessed in seedlings from both habitats. As expected, access to light induced a greater production of CBSCs in seedlings of both tree species, but these compounds did not seem to play a significant defensive role, as seedlings grown in gaps suffered greater leaf damage than those planted in forest interior. In addition, in both habitats, seedlings without insecticide treatment suffered a greater foliar damage than those with insecticide, especially 16 months after the beginning of the experiment. Canopy openness and herbivory had positive and negative effects, respectively, on seedling growth and survival in both tree species. In conclusion, despite the higher levels of defence in tree-fall gap, the higher densities of herbivore override this and lead to higher damage levels.

Keywords Canopy openness · Carbon-based secondary compounds (CBSCs) · Herbivory index (HI) · Relative growth rate (RGR) · Seedling survival

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Introduction

The most studied factor with regard to its effect on growth and survival of tree seedlings is light (Whitmore 1975, 1978; Denslow and Hartshorn 1994), which is likely to be more limiting than nutrients in most closed canopy rainforests (Denslow et al. 1998). In fact, "growth suppression" is a well-known phenomenon among seedlings and juveniles of shade-tolerant tree species. Growth suppression may be interpreted as a mechanism that allows seedlings to survive under poor light in the forest understory over long periods (Nava and del Amo 1985). Seedling banks represent a significant part of the regeneration potential of forests following canopy disturbance (Gómez-Pompa and Vázquez-Yanes 1985).

On the other hand, herbivory has been described as a critical biotic factor for growth and survival of newly established tree seedlings or juveniles (Connell 1971; Armstrong and Westoby 1993; Crawley 1997), particularly in light-limited environments (Núñez-Farfán and Dirzo 1985). In fact, numerous studies have documented strong effects of insect herbivory on the growth, reproduction, and survival of plants (see reviews by Louda et al. 1990; Huntly 1991) both in tropical forests (e.g. Becker 1983) and in temperate ones (e.g. Payne and Maun 1984; Bach 1994). Then, if herbivory reduce

seedling performance, the presence of secondary metabolites as defence mechanisms against a wide array of herbivores should be a primary strategy to reduce foliar damage.

For more than 30 years, carbon-based secondary compounds (CBSCs), such as phenols or tannins, have been considered as one of the most general chemical barriers of woody plants against herbivores (Feeny 1970; Bryant et al. 1987). Accordingly, the world is green because plant secondary metabolites generally make them poor food for herbivores (Hartley and Jones 1997). Several hypotheses have been advanced to account for the variability in resource allocation to defensive compounds among plants growing in different sites (Berenbaum 1995; Stamp 2003). One of the most popular ones, the carbon-nutrient balance hypothesis (CNBH) (Bryant et al. 1983), proposes that the carbon-nutrient status of a plant, as determined by habitat-related differences in light or nitrogen availability, directly controls the allocation to secondary metabolites, then the phenotypic expression of that plant's genetic potential for defences will be affected (see recent review by Stamp 2003). Therefore, plants in high light or nutrient-poor environments should allocate their excess photosynthates to carbon-based defences, thus reducing their susceptibility to herbivores (Bryant et al. 1987; Harborne 1997).

Denslow et al. (1990), in a tropical forest, and Dudt and Shure (1994), in a temperate forest, found that tree seedlings produced more CBSCs and suffered less foliar damage when growing under tree-fall gaps (light-rich habitat) than under a closed canopy (light-poor habitat). However, reduced foliar damage in light-rich habitats can also be a consequence of differences in herbivore abundance. Although some evidence suggests that insect herbivores may be more active in the forest understory than under tree-fall gaps (Newbery and de Foresta 1985; Denslow 1987; Calvo-Irabién and Islas-Luna 1999; Shure and Philips 1991), other studies have reported the opposite (e.g. Sipura and Tahvanainen 2000). Denslow et al. (1990) and Dudt and Shure (1994) also found that relative growth rates (RGR) and survival of tree seedlings were higher in tree-fall gaps than in the forest understory, in part, as a consequence of lower foliar damage. Nevertheless, as insect herbivores were not controlled, it cannot be completely ascertained whether higher seedling growth and survival in open areas resulted simply from greater access to light (the primary limiting factor for growth in many forests), from reduced foliar damage, or from the combination of both factors.

Here, we propose an experiment that considers the exclusion of insect herbivores to assess whether differences in seedling performance between open and shaded habitats are causally related to increased leaf concentration of CBSCs functioning as anti-herbivore defences. This experiment can also answer whether access to light and foliar damage have distinct or combined effects on seedling growth and survival. For this, we compared the growth and survival of young seedlings of two relatively shade-intolerant tree species or 'nomads' (sensu Martínez-Ramos 1985, see also Clark 1994). Juvenile stages of these trees can survive several years in shaded habitats in temperate rainforests (Armesto and Figueroa 1987; Aravena et al. 2002), but require small to medium-sized tree-fall gaps to reach the canopy. We addressed the following three questions about the interactions between light availability, plant chemistry and herbivory: (1) does canopy openness affect the production of CBSCs in tree seedlings?, (2) do CBSCs reduce foliar damage caused by insect herbivores?, and (3) how do canopy openness and foliar damage affect seedling growth and survival in a temperate rainforest?

To answer these questions, we designed a two-way factorial experiment, with light environment (forest interior vs tree-fall gap) and insecticide treatment (with or without insect herbivores) as factors affecting growth and survival of seedlings of *Drimys winteri* J. R. et G. Frost (Winteraceae) and *Gevuina avellana* Mol. (Proteaceae) in a temperate rainforest of Chiloé Island, southern Chile. These species are frequently represented in seedling banks (Armesto and Figueroa 1987; Aravena et al. 2002), but the effect of insect herbivores on their growth and persistence is poorly understood.

Materials and methods

Study site

The study was conducted in a large (> 100 ha) remnant patch of temperate rainforest in a rural area of northeastern Chiloé Island, about 20 km south-east of Ancud (41°53′S) at 80 m elevation (Fig. 1). The prevailing climate is wet-temperate with a strong oceanic influence (di Castri and Hajek 1976). Meteorological records at Senda Darwin Biological Station (41°51′S), less than 10 km from the study site, indicate an average annual rainfall of 2,120 mm, mean minimum temperature 3.5°C, mean maximum 14.3°C, and mean annual temperature 8.9°C (Aravena et al. 2002). The forest fragment is representative of the extensive primary forests that once covered the island landscape (Darwin 1859; Willson et al. 1994; Willson and Armesto 1996). The forest canopy was dominated by evergreen broad-leaved trees, such as *Laureliopsis philippiana*, *D. winteri*, *Amomyrtus luma*, *A. meli* and *G. avellana*, with occasional emergents (> 30 m tall) of *Eucryphia cordifolia* and *Nothofagus nitida*. The vegetation surrounding the patch consists mostly of open pasture for livestock. Some medium to large-sized canopy gaps (110–210 m^2), most of them due to tree falls, occur within the patch. The understory vegetation is sparse due to the dark understory, except under tree-fall gaps where a dense cover of native bamboos develops. Tree regeneration in this type of forest is strongly dependent on seedling banks and gap formation (Veblen and Alaback 1996; Veblen et al. 1996; Donoso 1989; Veblen 1985).
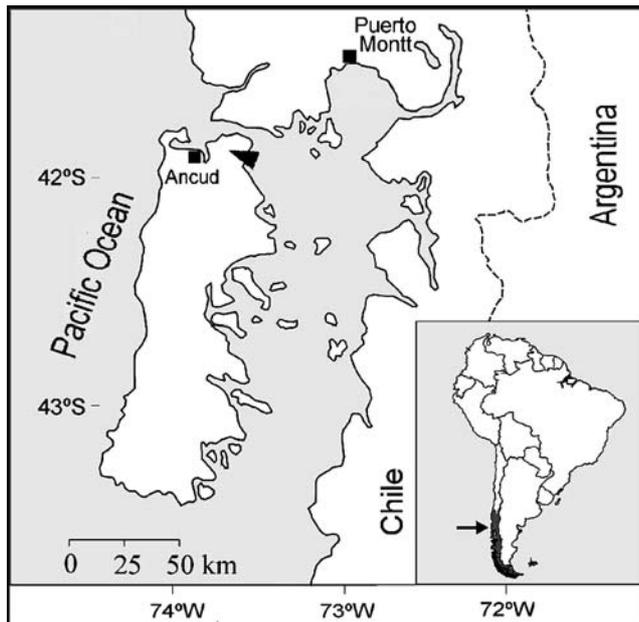
Fig. 1 Location of the study site in northern Chiloé Island, southern Chile. The *arrow* indicates the geographical position of Senda Darwin Biological Station and the study site. *Inset* shows the geographic distribution of South American temperate rainforests (*shaded*)

Tree species

Two tree species; *D. winteri* (Winteraceae) and *G. avellana* (Proteaceae) were the experimental subjects. Both species can reach up 25 m tall and have been described as intermediate in their shade tolerance, requiring medium-sized tree-fall gaps to become established (Donoso 1989; Veblen and Alaback 1996). Recently, Figueroa and Lusk (2001) reported that mean seedling light requirements (MSLR) for *D. winteri* and *G. avellana* seedlings were 8.8 and 6.6%, respectively, indicating that both species can grow and survive under low light levels. MSLR values for Valdivian rainforest tree species (Figueroa and Lusk 2001) ranged between 2.4% (*Myrceugenia planipes*) and 25.6% (*Embothrium coccineum*).

Habitats

We compared two forest habitats with contrasting light availabilities: (1) forest interior under a closed canopy, at least 100 m away from the nearest forest edge, and (2) tree-fall gap: inside an opening of the canopy created by multiple tree falls in the last 3–5 years, mean area = 150 m^2. Light environments in the two sites were quantified using two LAI-2000 Canopy Analysers (Li-Cor, Lincoln, NE, USA). Percentage canopy openness above a given point was determined by comparing two simultaneous LAI-2000 readings, one inside the forest patch or under a canopy gap within the patch, and a second one in an open area of pasture outside the

forest. LAI-2000 readings are a good surrogate of variation in total daily photosynthetic photon flux density (PPFD) in forest understory environments (Machado and Reich 1999; Figueroa and Lusk 2001). Mean percentage of canopy openness was 11.5% ± 1.1 for tree-fall gap, but only 1% ± 0.2 for locations under the closed canopy (mean ± 1 SE, $n = 10$ records per habitat). These values are comparable with those found in evergreen forests of La Selva Biological Station, Costa Rica (Denslow et al. 1990). We found no differences in soil moisture or total inorganic nitrogen (nitrate plus ammonium), measured at the beginning of the experiment and 6 months later, between canopy gap and forest interior (Chacón and Armesto 2005).

Experimental design and measurements

Seedlings of each species were grown for 2 months in a greenhouse from seeds collected from trees in the same forest patch where the experiment was conducted. After germination, each seedling was transplanted to an individual 20×20 cm^2 plastic pot filled with sieved topsoil and was acclimated for 1 month outside the greenhouse at Senda Darwin Biological Station before moving them to the experimental sites. In the forest interior and under tree-fall gap, we placed pots containing seedlings at ten randomly chosen points (separated about 5 m from one another). In each point, we placed four seedlings of *D. winteri* and four of *G. avellana* (eight seedlings per point, $n = 40$ seedlings per species per habitat). Pots were almost completely buried into the ground at each experimental site. Before the experiment was initiated, each seedling was individually tagged and measured (height and any signs of herbivory). Initial shoot length was 3.0 ± 0.1 cm for *D. winteri* (mean ± 1 SE) and 4.9 ± 0.1 cm for *G. avellana*.

To exclude insect herbivores, the most significant herbivores in this forest (P. Chacon, personal observation), half of the seedlings in each habitat were irrigated with Dimetoate *plus* + piretrine ®, a wide-spectrum systemic contact insecticide, manufactured by Agrícola Nacional, Santiago, Chile (ANASAC). Active ingredient of this insect repellent (dimetoate + piretrine) has a time of action of ≈25 days and is effective to control a wide array of chewing and sucking insects. Every month, each seedling was sprayed with 25 cc of insecticide at a dose of 10 cc per litre of water. There are no evidences that the components of this insecticide have any effects on seedling performance (ANASAC, personal communication). The other half of the seedlings (as control) was irrigated with a similar volume of water. Lambdon and Hassal (2001) suggested that insecticides operate in a similar way as secondary metabolites in many plant species making them a useful experimental tool.

Seedling growth, survival, and foliar damage attributable to insect herbivores were monitored twice a month, during a period of 16 months (December 2001–April 2003). Total shoot length (i.e. primary and lateral

shoots) was measured using a vertical ruler, and the number of fully expanded leaves per seedling was recorded.

Herbivory

The magnitude of herbivory was assessed as the percentage of leaf area removed. Damage imputable to defoliating insects was recorded, but we did not consider as insect damage, the chlorotic and necrotic spots that may suggest attack by pathogens or micro-organisms (Nava and del Amo 1985). In each seedling, leaves were assigned to a category of damage according the leaf area removed: $0 = 0\%$, $1 = 1–15\%$, $2 = 15–50\%$ and $3 = 50–100\%$. These categories are a simplification of those proposed by Núñez-Farfán and Dirzo (1985). An index of herbivory (IH), based on leaf damage scores, was defined as: $IH = \Sigma\, C_i \times n_i/N$, where C_i corresponds to the midpoint of each damage category (i.e. $C_1 = 8$, $C_2 = 33$, and $C_3 = 75\%$, respectively), n_i is the number of leaves per seedling in the ith category of damage, and N is the total number of leaves in each seedling (modified from Dirzo and Domínguez 1995; see also Boege and Dirzo 2004). This analysis makes it possible to distinguish between insect sampling, represented by low levels of foliar damage, and true insect feeding. In addition, this method of evaluating leaf damage is non-destructive, fast and accurate (Rodríguez-Auad and Simonetti 2001).

Growth and survival

Shoot lengths were used to calculate RGR using the equation: $RGR_s = (\ln\, H_{t2} - \ln\, H_{t1})/(t_2 - t_1)$, where H is seedling height (in cm) at t_1, the time when the experiment was set up, and at t_2, the end of the growing season (Hunt 1982; Denslow et al. 1990). Although, dry mass is the best parameter for estimating RGR, we used seedling height because it is not destructive and plants showed no signs of etiolation. Mortality was recorded when we observed the senescence and fall of all leaves.

Determination of total phenols and condensed tannins

To analyse the relationship between insect damage and foliar concentration of total phenols and condensed tannins, we collected 20 fresh leaves per habitat per species (one leaf per seedling) two times in a year: winter (June 2002) and summer (February 2003). Leaves were bagged in the field and refrigerated within 2 h of collection. Collected leaves were similar in appearance and position in the shoot. Samples were oven-dried at 35°C for 48 h and weighed. The low drying temperature prevented denaturing of phenolic compounds (Swain 1979; Hagerman 1988). Dried samples were ground to powder using a mortar and 50 mg of each leaf were extracted using 50% (v/v) methanol at 95°C (Bate-Smith 1977). Total phenols and condensed tannins were assayed using the Folin-Denis (Swain and Hillis 1959) and Butanol–HCl techniques (Bate-Smith 1977), respectively. Total phenols were expressed in tannic acid equivalents (as a percentage of leaf dry mass; % TAE) and condensed tannins as percent Quebracho tannin equivalents (% QTE). Tannic acid as well as Quebracho standards were obtained commercially. Both phenols and condensed tannins are suitable for testing the predictions of CNBH because both are products of the shikimate synthesis pathway (Lerdau 2002).

Biomass of insect herbivores

To determine potential differences in the biomass of insect herbivores between forest interior and tree-fall gap, we collected insects from each habitat during summer (December 2002) using two methods; pitfalls and vacuum sampling. These techniques allowed the collection of insects associated with litter and understory vegetation. In each of ten points per habitat, we buried a 500 cc pot containing formalin and left it for 48 h. For the second method, we shook at least ten juveniles of *Drimys* and ten of *Gevuina* (< 2 m height) per habitat over a white sheet and then vacuumed all insects within a 0.25 m² plastic cylinder (7 cm high), covered with netting to prevent insect escape. Collected insects from both sampling methods and habitats were classified in herbivores and non herbivores, placed in a drying oven at 75°C for 72 h and weighed to the nearest 0.1 mg.

Statistical analyses

A repeated measures analysis of variance (rANOVA) assessed differences in concentration of both types of CBSCs among leaves of seedlings growing in different habitats (forest interior vs tree-fall gap). To detect habitat and insecticide treatment effects on HI, we also used a rANOVA considering three response variables: level of foliar damage at the beginning of the experiment, after 8 months (August 2002) and after 16 months (April 2003). A posteriori Student Newman–Keuls tests were performed for comparing among treatments. Differences in RGR were tested using a two-way analysis of variance (ANOVA) with habitat and insecticide treatment as factors, and a posteriori Tukey's test for comparisons among treatments. Finally, to test for differences in seedling survival, we used a "Binomial Lineal Model with Logit" (Statistica, StatSoft Inc. 1998), a method based in a logistic regression that allows the evaluation of possible effects of two or more factors and their interaction over a discrete variable (number of surviving seedlings). The factors considered in this analysis were habitat, insecticide treatment and time (number of months after initiation of the experiment). In addition, the final number of seedlings that remained

alive at the end of the experiment in each treatment was compared using a multiple comparisons test of proportions and a posteriori Tukey's test (Zar 1996). The number of replicates per habitat per species was 20 for each response variable. Finally, we determined whether the final number of dead seedlings that showed foliar damage (herbivory index greater than zero) differed from that expected by chance using a Chi-squared test (Zar 1996).

Results

Carbon-based secondary metabolites

Concentrations of foliar phenolic compounds in seedlings of both tree species was affected by light environment (forest interior vs tree-fall gap) (ANOVA with repeated measures, $F_{Phenols} = 21.67$; $df = 2$, 39; $P < 0.001$ and $F_{Tannins} = 8.79$; $df = 1$, 25; $P = 0.006$ for *D. winteri* and $F_{Phenols} = 8.24$; $df = 1$, 19; $P = 0.009$ and $F_{Tannins} = 17.51$; $df = 1$, 39; $P < 0.001$ for *G. avellana*). A higher concentration of foliar phenolic compounds was found in seedlings growing in tree-fall gap (a posteriori Student Newman–Keuls tests, $P < 0.05$; Fig. 2). We also observed strong seasonality in the production of total phenols in seedlings of *D. winteri* in tree-fall gap (a posteriori Student Newman–Keuls tests, $P < 0.05$; Fig. 2a). Phenolic compounds concentrations ranged between 2 and 22% per mg of dry leaf in both species, and as expected, tannins were lower than total phenols, as they are a subset of total phenols (Fig. 2).

Leaf damage by insects

Habitat differences had significant effects on herbivory index in seedlings of both species, while insecticide treatment significantly reduced insect herbivory in *D. winteri* but only marginally in *G. avellana* (Table 1). At the time of transplant, seedlings of *D. winteri* and *G. avellana* that were randomly allocated to each habitat or insecticide treatment did not differ in herbivory levels (a posteriori Student Newman–Keuls tests, $P > 0.05$). Eight months later, herbivory index increased in both species, but no differences were detected between plants treated with insecticide and control plants (Student Newman–Keuls tests, $P > 0.05$) (Fig. 3a, b). Finally, 16 months later, control plants (without insecticide) of both species growing in tree-fall gap showed greater leaf damage, differing significantly from the other three habitat–insecticide combinations (Student Newman–Keuls tests, $P < 0.05$) (Fig. 3c, d). Thus, control (without insecticide) seedlings of *D. winteri* and *G. avellana* in tree-fall gap suffered 2.8 and 2.9 times greater levels of herbivory respectively, than those in the forest interior with insecticide (Fig. 3c, d). The final herbivory index (% lost leaf area) after 16 months ranged between 2.3 and 16.5 in both tree species and across all habitats and treatments.

Seedling growth and survival

Habitat differences (forest interior vs tree-fall gap) and insecticide treatment (with and without insecticide) had

Fig. 2 Percentage of total leaf phenols and condensed tannins in *D. winteri* (**a**, **c**) and *G. avellana* (**b**, **d**) seedlings grown under closed canopy (forest interior) and under light gap. Leaves were collected in winter (*grey bars*) and summer (*open bars*). Means ± 1 SE are shown. Sampling date was considered a repeated measure in the ANOVA
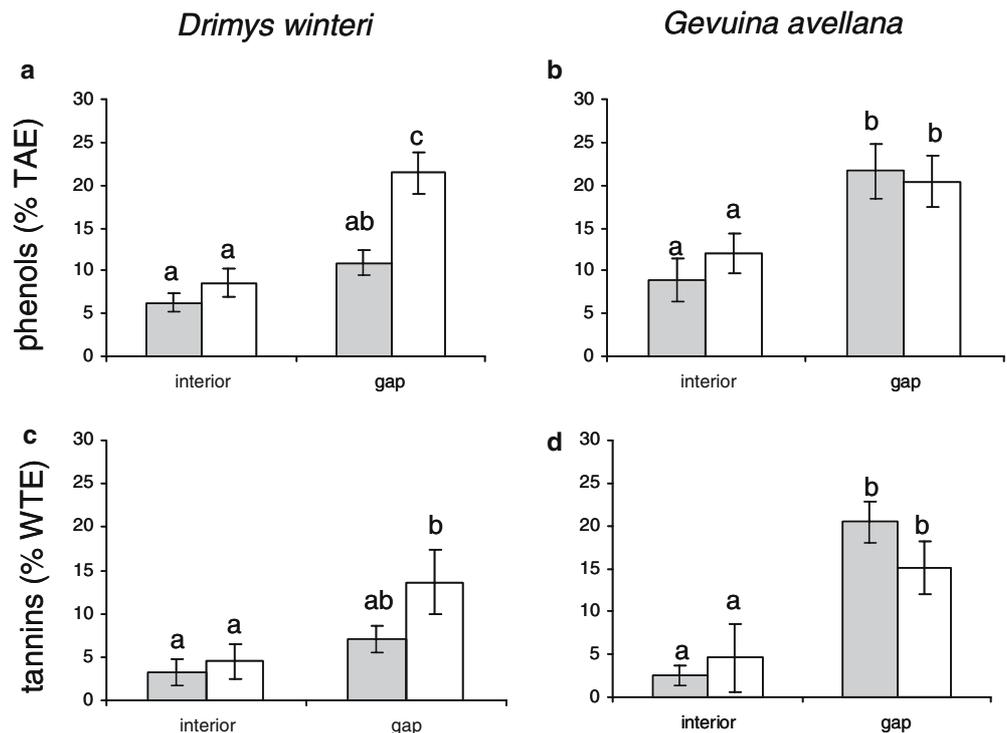
Table 1 Repeated measures ANOVA evaluating the effect of habitat (forest interior vs tree-fall gap) and insecticide treatment (with or without) on herbivory index (% leaf area lost)

Source	df	MS	F	P
Drimys winteri				
Between subjects				
Habitat (H)	1	623.146	19.272	0.001
Insecticide (I)	1	140.862	4.356	0.040
H×I	1	22.530	0.696	0.406
Error	76	32.333		
Within subjects				
Time (T)	2	2191.141	74.716	0.001
T×H	2	251.583	11.505	0.001
T×I	2	71.063	3.249	0.041
T×H×I	2	14.780	0.675	0.510
Error	152	0.4362		
Gevuina avellana				
Between subjects				
Habitat (H)	1	356.399	14.447	0.001
Insecticide (I)	1	89.430	3.625	0.060
H×I	1	39.542	1.602	0.209
Error	76	24.669		
Within subjects				
Time (T)	2	828.697	49.998	0.001
T×H	2	112.522	6.788	0.001
T×I	2	26.464	1.596	0.205
T×H×I	2	15.827	0.954	0.387
Error	152	16.575		

significant effects on seedling RGR_s in both species ($F_{Habitat} = 16.27$; $df = 1$, 66; $P < 0.001$ and $F_{Insecticide} = 4.35$; $df = 1$, 66; $P = 0.04$ for *D. winteri* and $F_{Habitat} = 59.35$; $df = 1$, 73; $P < 0.001$ and $F_{Insecticide} = 10.08$, $df = 1$, 73; $P < 0.002$ for *G. avellana*). In addition, a significant interaction between both factors was detected in *G. avellana* ($F_{Insecticide \times habitat} = 5.58$; $df = 1$, 73; $P = 0.020$). RGR_s of insecticide-treated seedlings of both species in tree-fall gap were higher (0.1077 and 0.0856 cm cm^{-1} month^{-1} for *D. winteri* and *G. avellana*, respectively) than seedlings from forest interior, with or without insecticide (a posteriori Tukey test, $P < 0.05$) (Fig. 4a, b). Moreover, RGR_s of control seedlings of *G. avellana* grown in tree-fall gap differed from those grown in forest interior treated or not treated with insecticide (a posteriori Tukey test, $P < 0.05$) (Fig. 4b). Finally, RGR_s differed between *G. avellana* seedlings with and without insecticide application when growing under a tree-fall gap (a posteriori Tukey test, $P < 0.05$) (Fig. 4b).

Habitat, insecticide application and time (number of months after the experiment was initiated) had significant effects on seedling survival in both tree species

Fig. 3 Herbivory index (± 2 SE) in seedlings of *D. winteri* (**a**) and *G. avellana* (**b**) grown under closed canopy (forest interior) and under light gap in August 2002 (8 months after the experiment was initiated) and in April 2003 (16 months later). *Shaded bars* insecticide treated plants; *open bars* control plants. *Bars with different letters* differ significantly (a posteriori Student Newman–Keuls test, $P < 0.05$)

Drimys winteri

Gevuina avellana
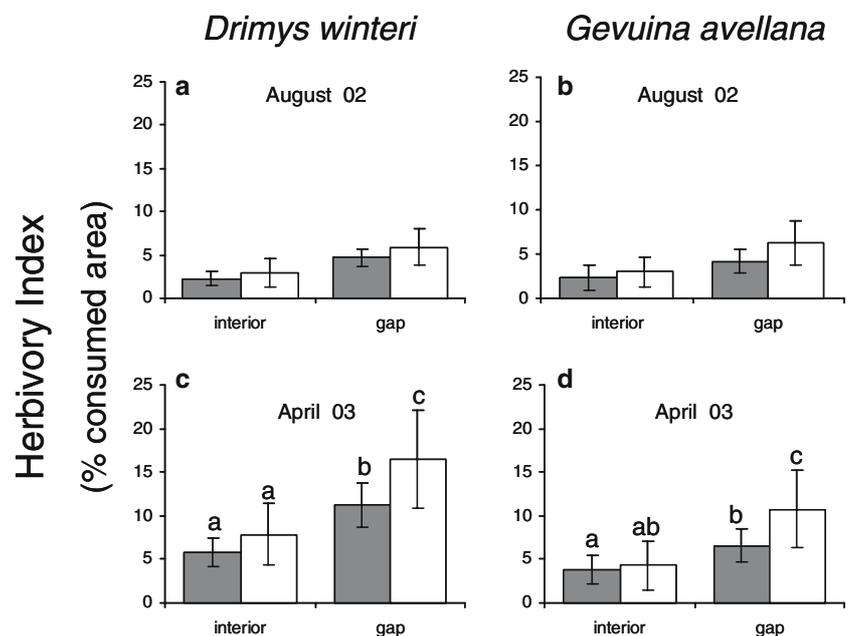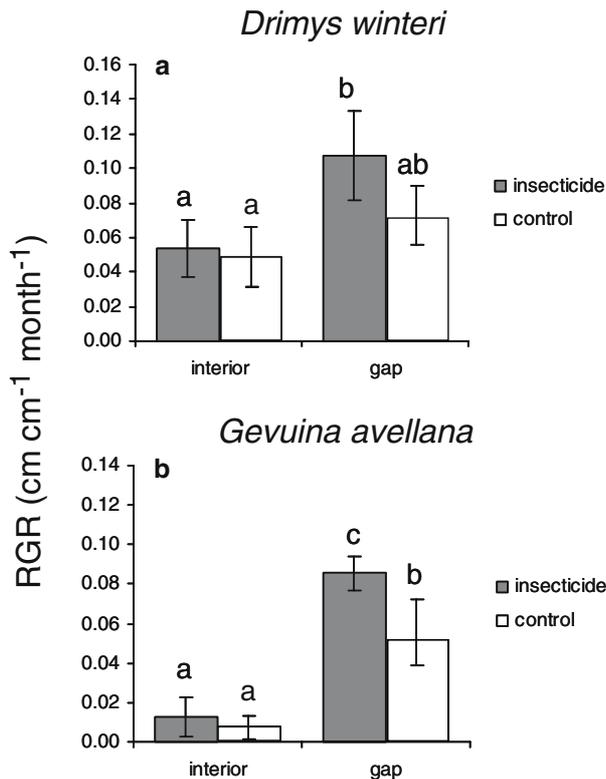
Fig. 4 Relative growth rate of shoots ($RGR_s \pm 2$ SE) in seedlings of *D. winteri* (**a**) and *G. avellana* (**b**) grown under closed canopy (forest interior) and under light gap during a period of 16 months (December 2001–April 2003). *Shaded bars* insecticide treated plants; *open bars* control plants. *Bars with different letters* differ significantly (a posteriori Tukey test, $P < 0.05$)

(Table 2). Significant interactions between habitat and insecticide application and between insecticide treatment and time were detected in the case of *G. avellana* (Table 2). Insecticide-treated seedlings of both tree species survived better in tree-fall gap during the study period (Fig. 5a, c). Seedling survival was particularly reduced in *G. avellana* in forest interior, especially after the first 6 months (Fig. 5c). At the end of the experiment, the percentage of surviving control seedlings of *D. winteri* in forest interior was significantly lower than in tree-fall gap, with or without insecticide (a posteriori Tukey-type's tests for multiple comparisons, $P < 0.05$) (Fig. 5b). Seedlings of *G. avellana* showed a similar trend, but their overall survival was lower (Fig. 5d).

The proportion of dead seedlings that suffered herbivore damage (herbivory index greater than zero) differed significantly from that expected by chance alone for control (without insecticide) seedlings of both tree species grown in forest interior and in tree-fall gap (Chi-squared tests, $P < 0.05$) (Table 3).

Biomass of insect herbivores

The biomass of insect herbivores from pitfall traps, expressed as a percent of total biomass of insects, differed between forest interior (10.5% ± 5.0; mean ± 1 SE) and tree-fall gap (27.8% ± 8.4) (Student's test, $t = 2.04$; $df = 18$; $P < 0.05$). Grasshoppers (Acrididae–Orthoptera) were the main herbivores found through this technique. On the other hand, herbivore biomass estimated from sweeping method was greater than from pitfall traps, ranging between 84.3% in forest interior and 96% in tree-fall gap. With this method, Lepidopteran larvae, mainly those in the Family Geometridae, were the main herbivores.

Discussion

We investigated two main questions. First, whether the light environment affects foliar concentrations of phenolic compounds in seedlings of two rainforest tree species, and whether these compounds, if present, play a defensive role against insect herbivores. The second question was whether habitat-related differences in light environment and foliar damage affected growth and survival of tree seedlings.

CBSC and their potential defensive role

The observed concentrations of phenolic compounds in seedlings supported the predictions of the carbon–nitrogen balance hypothesis (CNBH), as seedlings of both species in tree-fall gap had a greater foliar concentration of phenols and condensed tannins. However, seedlings in tree-fall gap did not show a significant reduction in the levels of foliar damage due to insect herbivores, compared to seedlings in the forest interior. On the contrary, seedlings of both species suffered significant more leaf damage in tree-fall gap than in the shade. This result is partly consistent with findings of Denslow et al. (1990), Shure and Wilson (1993), and Dudt and Shure (1994). Denslow et al. (1990), for instance, found that a greater concentration of phenols in seedlings of *Miconia barbinervis*, inside tree-fall gaps reduced the levels of herbivory relative to seedlings under closed canopy, but seedlings of *Piper culebranun* and *P. urostachyum* were more damaged by herbivores in tree-fall gaps despite their greater concentration of phenols. For other species (*M. nervosa*, *M. gracilis*, *P. sancti-felicis* and *P. arieianum*) these authors did not find a relationship between phenolic compounds and foliar damage.

These conflicting results may be explained by habitat-related differences in variables, such as temperature, that may affect insects and, consequently herbivory levels (Moore et al. 1988; Weis et al. 1988). Sipura and Tahvanainen (2000) showed that two willow-feeding leaf beetles were more abundant on potted willows growing in open habitats than on the same willow clones placed under tree shade. However, at constant temperature in the laboratory, both adults and larvae preferred to feed on shaded willows. These authors suggested that open habitats offer more favourable conditions in the field,

Effect	df	Log-likelihood	χ^2	P
Drimys winteri				
Intercept	1	−381.388		
Habitat (H)	1	−343.989	74.798	0.001
Insecticide (I)	1	−335.262	17.455	0.001
Time (T)	8	−318.458	33.607	0.001
H×I	1	−318.213	0.490	0.483
H×T	8	−316.124	4.178	0.840
I×T	8	−315.574	1.098	0.997
H×I×T	8	−315.479	0.190	0.999
Gevuina avellana				
Intercept	1	−451.044		
Habitat (H)	1	−392.750	116.587	0.001
Insecticide (I)	1	−390.569	4.361	0.036
Time (T)	8	−291.177	198.785	0.001
H×I	1	−279.623	23.107	0.001
H×T	8	−273.368	12.509	0.129
I×T	8	−264.085	18.567	0.017
H×I×T	8	−263.088	1.993	0.981

which may override food quality as a determinant of choice. Other authors have suggested that shading may affect orientation behaviour and growth of herbivorous insects (Weis et al. 1988; Louda and Rodman 1996). In some cases, herbivores may use secondary compounds as cues to feed or reproduce. Some types of CBSCs (e.g. salicylates) are effectively used by insects as feeding or ovoposition cues (Tahvanainen et al. 1985). Finally, gap ages may affect insect abundance. For example, Maleret et al. (1978) found that insect abundance was highest in newly formed gap, intermediate in the "building stage" (see Whitmore 1984), and lowest in the understory of closed canopy forest. These different lines of evidence suggest that seedlings inside tree-fall gaps may generally

be exposed to more intense insect herbivory regardless of the presence of greater concentrations of defensive compounds. In fact, our results indicate a greater biomass of insect herbivores in tree-fall gap than in forest interior, estimated by two collecting techniques, and a lower foliar C/N ratio in seedlings of both species grown in gap (data not shown). Consequently, despite the higher levels of phenolic compounds in gaps, the higher densities of herbivores in this habitat may potentially lead to higher damage levels and therefore, plants growing in gaps could suffer even more damage if they did not increase their defence levels.

Recently, phenolic compounds have been associated with other functions unrelated to protection from her-

Fig. 5 Percentage of seedlings of *D. winteri* (a, b) and *G. avellana* (c, d) grown under closed canopy (forest interior) and under light gap that survived after 16 months. *Grey symbols* insecticide treated plants, *open symbols* control plants, *circles* plants located in forest interior, *squares* plants located in tree-fall gap. *Shaded bars* insecticide treated plants, *open bars* control plants. *Bars with different letters* differ significantly (a posteriori Tukey test, P < 0.05)
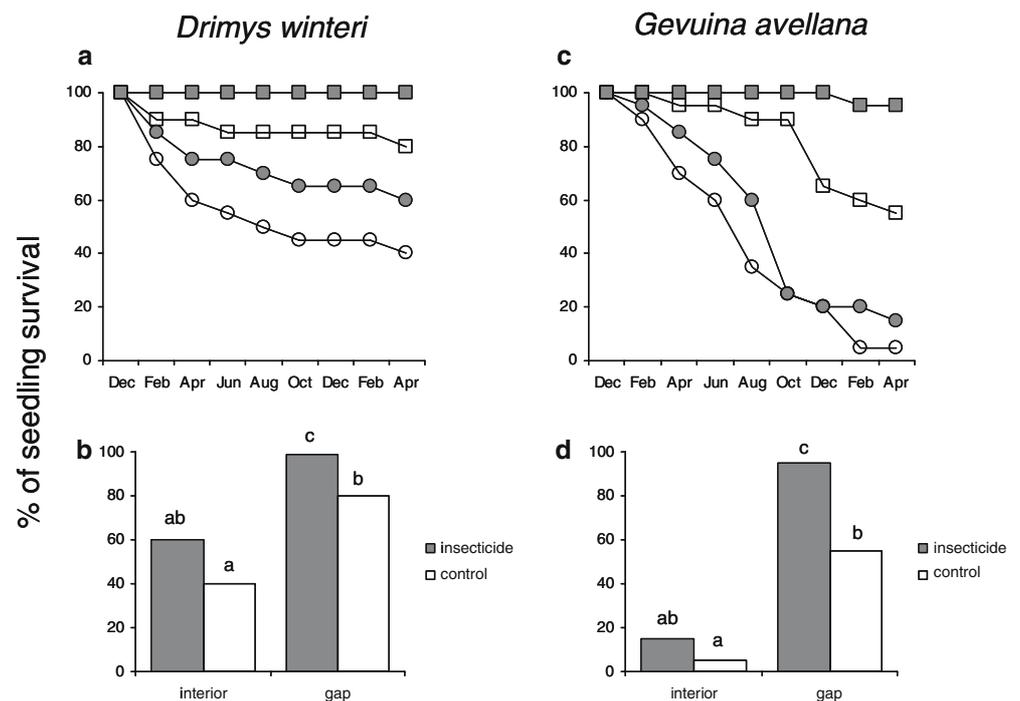
Table 3 Number and percentage of dead seedlings of *D. winteri* and *G. avellana* that showed foliar damaged (herbivory index greater than zero)

Asterisks indicate statistically significant differences indicating that the proportion of dead seedlings with damage differed from that expected by chance ($P < 0.05$)

Habitat	*Drimys winteri*			*Genuina avellana*		
	Dead and damaged seedlings	Total dead seedlings	% dead and damaged	Dead and damaged seedlings	Total dead seedlings	% dead and damaged
Interior						
Insecticide	6	8	75.0	10	17	58.8
Control	10	12	83.3*	15	19	78.9*
Tree-fall gap						
Insecticide	0	0	0.0	1	1	100.0
Control	4	4	100.0*	11	13	84.6*

bivory. Close and McArthur (2002) attributed them a role in protecting leaves from photodamage, acting as antioxidants. There is an increasing evidence that a range of phenols has antioxidant capacity and may protect plants from photodamage depending on light conditions. Antioxidant activity has been demonstrated in vitro for flavonoids (Gardner et al. 1998), anthocyanins (Wang et al. 1997) and also for condensed and hydrolysable tannins (Hagerman et al. 1998). Accordingly, concentrations of these phenols are expected to be low under low light not because resources to produce them are limited, but simply because of a lower risk of photodamage. If phenols protect plants from ultraviolet radiation (see also Waterman et al. 1984), our current understanding of plant defence theory needs rethinking. In our study, the greater concentrations of foliar phenolic compounds found in tree seedlings of both tree species when growing in a light gap is consistent with Close and McArthur's (2002) hypothesis.

Effects of canopy openness and herbivory on seedling performance

Through the insecticide treatment, we evaluated the combined effects of light environment and insect herbivory on growth and survival of tree seedlings. We found that light environment (tree-fall gap vs forest interior) and foliar damage had significant effects on seedling growth and survival of both tree species. Although the effect of light environment on seedling growth has been well documented, few field studies have reported increased seedling mortality as a consequence of herbivory or combined effects between both factors. In examining the interactions between light and herbivory, and in contrast to our prediction, the greatest difference in growth and survival between tree seedlings with and without insecticide treatment was recorded in the light gap where a greater herbivore pressure was observed. In other words, it means that protection from herbivores had a bigger effect in tree-fall gap. In synthesis, although insecticide application was not fully successful in reducing foliar damage, this small difference was sufficient to increase the RGR of shoots and the overall survival of treated seedlings.

Growth and survival of seedlings of both tree species was always greater under tree-fall gap and lower in the forest interior, despite the fact that seedlings in light gap exhibited greater levels of foliar damage. In contrast, although we observed relatively low levels of herbivory in the understory; both growth and survival were less in this habitat, implying that light was a more limiting factor for plant performance. We predicted that the impact of herbivory on seedling growth and survival should be lower in open habitats, because the lower cost of replacing foliar tissue losses (Denslow 1987; Denslow and Hartshorn 1994). While in the tree-fall gap seedlings of both tree species produced an average of six new leaves, in the forest interior the increment did not reach two leaves in a period of 16 months (Chacón and Armesto 2005). A growth reduction in the forest interior, as observed in this study, is even more marked in the neotropical tree *Dipteryx panamensis* (Clark and Clark 1984). Seedlings growing in the understory are unable to form new leaves during the first 7 months after germination. This evidence suggests that detrimental effects of herbivory are greater when they are combined with light stress (Núñez-Farfán and Dirzo 1985).

One important result of this study is the experimental demonstration that foliar damage caused by insect herbivores, even at low levels, reduces growth and survival of tree seedlings. Although, many studies have shown negative effects of herbivory on seedling performance (e.g. Marquis 1984; Coley 1986; Osunkoya et al. 1993; Terborgh and Wright 1994; Ickes et al. 2001; Wallin and Raffa 2001), few of these have focused on insect herbivory (Jackson and Bach 1999). In our study, one-third of the mortality of *D. winteri* seedlings in the forest interior could be attributed to insect damage. This value may be underestimated considering that the insecticide treatment was not fully successful in preventing insect damage. Clark and Clark (1985) found that annual survivorship was 85% for undamaged seedlings of *D. panamensis* and 0% for seedlings with 8% of their leaf area missing. Seedlings of *Nectandra ambigens*, *Omphalea oleifera* and *Brosinum alicastrum* suffered high mortality and growth reduction when subjected to herbivory under closed canopy (Dirzo 1984). In contrast, experimental defoliation did not affect seedling survival of *Shorea leprosula* and *S. maxwelliana* (Becker 1983). Recently, Pearson et al. (2003) found that herbivore exclusion reduced foliar damage, but herbivores only affected the survival of one out of three tree species studied. In *Trema micrantha* 63% of damaged seedlings died in a period of 2 months. Her-

bivory exclusion also increased RGR of seedlings of *T. micrantha* and *Miconia argentea*. These data suggest that herbivory by insects may be an important constraint on the persistence of seedling banks in closed canopy forests.

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