

Environmental and genetic control of insect abundance and herbivory along a forest elevational gradient

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Abstract Environmental conditions and plant genotype may influence insect herbivory along elevational gradients. Plant damage would decrease with elevation as temperature declines to suboptimal levels for insects. However, host plants at higher elevations may exhibit traits that either reduce or enhance leaf quality to insects, with uncertain net effects on herbivory. We examined folivory, insect abundance and leaf traits along six replicated elevational ranges in *Nothofagus pumilio* forests of the northern Patagonian Andes, Argentina. We also conducted a reciprocal transplant experiment between low- and high-elevation sites to test the extent of environmental and plant genetic control on insect abundance and folivory. We found that insect abundance, leaf size and specific leaf area decreased, whereas foliar phosphorous content increased, from low-, through mid- to high-elevation sites. Path

analysis indicated that changes in both insect abundance and leaf traits were important in reducing folivory with increasing elevation and decreasing mean temperature. At both planting sites, plants from a low-elevation origin experienced higher damage and supported greater insect loads than plants from a high-elevation origin. The differences in leaf damage between sites were twofold larger than those between plant origins, suggesting that local environment was more important than host genotype in explaining folivory patterns. Different folivore guilds exhibited qualitatively similar responses to elevation. Our results suggest an increase in insect folivory on high-elevation *N. pumilio* forests under future climate warming scenarios. However, in the short-term, folivory increases might be smaller than expected from insect abundance only because at high elevations herbivores would encounter more resistant tree genotypes.

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Introduction

Spatio-temporal environmental changes are expected to alter interactions between plants and insect herbivores (Bale et al. 2002; Tylianakis et al. 2008). Among several contributory factors, temperature has been singled out as a major driver of plant-insect interactions (Bale et al. 2002; Deutsch et al. 2008; Tylianakis et al. 2008). Three separate bodies of evidence support this proposition, including results from short-term manipulation experiments, observations along large-scale environmental gradients and trends in the plant fossil record (for a review, see Wilf 2008). To date, studies have focussed on either short-term

(plastic) or long-term (genetic) plant responses to temperature (Bale et al. 2002; Wilf 2008), but there has been little integration of processes operating at different time scales. One approach to integrate both scales of analysis would be to examine extant herbivory patterns in combination with the reciprocal transplant of host-plant genotypes along environmental gradients (Pennings et al. 2009).

Elevational gradients have the advantage that meaningful environmental changes occur over relatively short distances, thus reducing the confounding effects of dispersal, daylength, species pools and the biogeographic history of plant–herbivore interactions (Hodkinson 2005; Körner 2007). While elevation correlates with well-documented changes in abiotic conditions and plant physiology (Ohsawa and Ide 2008), relatively little attention has been given thus far to concomitant changes in insect herbivory. Among the studies conducted to date, some have shown decreased plant damage by insects with increasing elevation (Galen 1990; Kelly 1998; Suzuki 1998; Alonso 1999), whereas others have found the opposite trend (Erelli et al. 1998; Hagen et al. 2007). Herbivory patterns along elevation gradients would be driven by both insect population dynamics and host-plant trait variation in response to changing environmental conditions (Koptur 1985; Scheidel et al. 2003; Hodkinson 2005).

The magnitude of herbivory depends on herbivore abundance and per capita consumption, both of which can be affected by shifts in climatic conditions and plant traits with elevation. From an insect perspective, slow growth rates in physically harsh environments may extend developmental time and reduce survival and consumption rates (Whittaker and Tribe 1996; Hodkinson 1997; Williams 1999). From a plant perspective, low temperatures and a shorter growing season at higher elevations would result in increased leaf nutrient concentrations (Körner 1989) and thus plant quality to insects (Erelli et al. 1998; Suzuki 1998). However, a decrease in leaf nutritional value with elevation might be also expected, because at low temperatures growth tends to be constrained more than photosynthesis, and carbohydrates may accumulate in excess of growth and maintenance requirements, leading to higher levels of carbon-based secondary metabolites (Herms and Mattson 1992; Stamp 2003). In general, slow-growing plants should have better defences against herbivores (Stamp 2003; Fine et al. 2004), and some adaptations to abiotic stress (e.g. leaf toughness, trichomes) may decrease herbivory as well (Coughenour 1985; Coley 1987; Agrawal 2007). The effects of such sub-lethal plant defences can be complex, as they sometimes increase herbivore per capita consumption, while also decreasing insect numbers through delayed development and increased exposure to natural enemies and abiotic stress (Coley and Barone 1996; Williams 1999).

Elevation may determine important changes in the physical environment that can promote diversifying selection on plant populations located only a few hundred meters apart (Premoli 2003; Herrera and Bazaga 2008; Ohsawa and Ide 2008). Phenological differences with elevation may impose restrictions to gene flow, reinforcing the genetic differentiation of local populations within species ranges (Premoli 2003; Ohsawa and Ide 2008). Both genetically based and environmentally induced phenological changes may in themselves explain patterns of cumulative herbivory along elevation gradients. Intra-specific differences in phenology may influence leaf longevity and therefore exposure to herbivores (Mopper and Simberloff 1995), or they may alter the timing between folivore activity and leaf availability (Aizen and Patterson 1995). If spatial gradients of insect herbivory were explained mainly by differences in constitutive plant traits, then temporal environmental changes would have little impact on herbivory in the short term (Andrew and Hughes 2007), especially in long-lived plants. Reciprocal transplant experiments can be used to understand the relative importance of plant genotype and local environment as major determinants of herbivory along elevation gradients.

Plant genotype and environmental effects on herbivory are expected to vary among insect-feeding guilds. For example, endophagous insects, such as leaf miners and galls, are thought to have higher host specificity than external feeders (Basset 1992; Novotny and Basset 2005). Thus, endophagous guilds might show stronger responses than exophagous guilds to plant genotype variation along environmental gradients. On the other hand, endophagous insects are regarded as being less vulnerable than exophagous insects to weather, including variations in diurnal temperatures and moisture conditions, although the net effects from direct (e.g. insect desiccation) and indirect (e.g. plant-mediated) influences on particular feeding guilds are not straightforward (Huberty and Denno 2004; Sinclair and Hughes 2010). The quantification of endophagous versus exophagous guild responses to plant genotype and site conditions is central to understanding the drivers of insect herbivory along environmental gradients.

In temperate forests, the elevational decrease in temperature may be of special importance in imposing harsher conditions to both insects and trees, since insects may encounter suboptimal temperatures at high latitudes (Deutsch et al. 2008), and trees are more exposed to atmospheric temperature than lower stature life forms (Körner 2007). Southern beech (*Nothofagus pumilio*) forests in the Patagonian Andes offer a convenient system to examine the controls of herbivory along elevational gradients. In northwestern Patagonia, Argentina, *N. pumilio* forms extensive monospecific stands between 1,000 m a.s.l. and the treeline at 1,600 m a.s.l., making it possible to evaluate

the effects of elevation on tree folivory without the confounding influence of plant composition. The aims of this study were (1) to assess changes in insect abundance, leaf traits and folivore damage on *N. pumilio* along replicated elevational ranges over an area of 3,300 km² of the Patagonian Andes, and (2) to determine the relative importance of local environment and genetically based plant resistance as controls of herbivory. We conducted reciprocal transplants of *N. pumilio* saplings from low- and high-elevation sites and followed their fate for two growing seasons. We tested for environmental influences on insect folivory by comparing each tree population between contrasting elevations (site effect). Further, we evaluated genotypic differentiation in plant resistance to folivory by comparing folivory levels between conspecific populations from different elevations (origin effect).

Materials and methods

Study system

Nothofagus pumilio (lenga) is a deciduous tree that dominates high-elevation forests of southern Argentina and Chile between approximately 35° and 55°S latitude (Veblen et al. 1996; González et al. 2006). At the latitude of the study (40–41°S), most precipitation falls as rain and snow during the autumn and winter (March–September), before the main growing season (October–April). In this region, the Andes form an effective barrier to the westerly airflow, creating a pronounced rain shadow east of the continental divide. Mean annual precipitation varies from approximately 3,000 mm year⁻¹ on the western limit of the forest in Argentina to approximately 800 mm year⁻¹ on the easternmost limit of *N. pumilio* distribution (Barros et al. 1983). Within the elevational range of *N. pumilio*, mean annual temperatures vary from 5–6°C (approx. 1,000 m a.s.l.) to 3–4°C (approx. 1,500 m a.s.l.), decreasing by approximately 0.5°C every 100 m of elevation (Rush 1993). High-elevation forests experience shorter periods with frost-free soils and a higher percentage of snowfall than low-elevation forests (Barrera et al. 2000). Precipitation does not consistently change with elevation within the region and elevational range studied here.

Previous work revealed that there are substantial changes in the physiognomy and genetics of *N. pumilio* with elevation. The vegetative growth period is shorter in high-elevation sites due to delayed budbreak and accelerated leaf fall (Rush 1993). Tree growth rates decrease with elevation (Barrera et al. 2000). At low elevations, *N. pumilio* grows up to 30 m tall, attaining a diameter at breast height (DBH) of 80–120 cm, and can be up to 300 years old (Heinemann et al. 2000). Tree height and

DBH sharply decrease with elevation, with trees showing a shrub-like ('krummholz') growth form at high-elevation sites (Barrera et al. 2000). Dendroecological studies at high elevations have revealed that in mesic topographic positions and/or during wet climatic periods, warmer temperatures favoured radial growth of *N. pumilio*, whereas under xeric conditions/periods, cooler temperatures favoured tree growth, possibly due to an indirect effect on water deficits (Daniels and Veblen 2004, Srur et al. 2008). Flowering is delayed at higher elevations (Rush 1993), and Premoli (2003) reported restrictions to gene flow between *N. pumilio* populations occupying the highest and lowermost distributional belts within a mountain range. Common garden experiments have revealed substantial differences in genetically controlled plant traits between saplings from low- and high-elevation sites (Premoli and Brewer 2007; Premoli et al. 2007), with high-elevation plants exhibiting lower annual shoot growth and leaf size, and delayed budbreak and leaf expansion, relative to low-elevation plants (Premoli et al. 2007). Insect folivory patterns have been described only for low-elevation *N. pumilio* forests, with the results showing that leaf damage on juvenile and adult trees is tenfold higher in a dry/warm forest stand than in a wet/cold one (Mazía et al. 2004; Garibaldi et al. 2010).

Regional sampling

During the 2005–2006 and 2006–2007 growing seasons, we collected *N. pumilio* foliage along six elevational gradients located in different mountain ranges of the northern Patagonian Andes in Argentina [40–41°S, 71°W; see Electronic Supplementary Material (ESM) Fig. S1]. Mountains were selected to represent contrasting positions along the dominant west-to-east precipitation gradient at different latitudes, which allowed us to encompass the existing environmental heterogeneity within the northern distributional range of *N. pumilio* forests (Veblen et al. 1996; González et al. 2006). Study sites were located at Cerro Tronador (3,250 mm of mean annual precipitation; Barros et al. 1983) and Cerro La Mona (2,550 mm) on the west end of the precipitation gradient, at Cerro Pelado (1,500 mm) and Cerro Chall Huaco (1,800 mm) on the east end and at Cerro Bayo (1,900 mm) and Cerro López (1,800 mm) in the middle range of the gradient (ESM Fig. S1).

On each mountain, we sampled leaves of low-elevation forests [mean ± standard deviation (SD): 1,098 ± 139 m a.s.l.; SD for differences among mountains] where *N. pumilio* occurs as a tall canopy tree, those of forests at intermediate elevations (1,311 ± 79 m a.s.l.) and those of forests at high elevations (1,525 ± 81 m a.s.l.) where *N. pumilio* exhibits a shorter, bush-like morphology. At each site (3 positions × 6 mountains = 18 sites), we

selected 15 adult trees located at least 10 m apart along an approximately 200-m-long walking transect. From each tree, we harvested one branch with approximately 400 leaves using an extensible pole cutter. For each of these trees, the canopy was visually divided into three vertical layers, and sample branches were collected from the middle canopy layer, regardless of branch orientation. Sampling was performed in late March–early April, towards the end of each of two growing seasons (hereafter referred to as April 2006 and April 2007, respectively). Different tree individuals were selected for each sampling season. In the first year, we also sampled foliage during late December–early January (hereafter January 2006) because early leaf abscission of damaged leaves and the flush of new leaves after January could affect leaf damage estimates in the late season. The three positions within a mountain range were sampled during the same day, and the six mountains were sampled within 12 days (the sampling order of sites depended on logistic constraints and differed among sampling dates). Logistics prevented us from sampling Cerro Bayo in January 2006 (mid-elevation site) and April 2006 (all three elevations); thus, the total number of observations for our regional sampling was 50 instead of 54.

We measured the abundance of and damage produced by different leaf feeding guilds, including endophagous insects, such as leaf miners and gall formers, and exophagous insects, such as skeletonisers, chewers, pit feeders and sap suckers (Mazía et al. 2004; Garibaldi et al. 2010). The most conspicuous leaf miners in the system are small-sized Lepidoptera (e.g. Heterobathmiidae) and sawfly larvae (Hymenoptera, Symphyta); gall formers include gall-wasps [Hymenoptera: Cynipidae (e.g. *Paraulax* spp.) and Pteromalidae (e.g. *Aditrochus fagicolus*); McQuillan 1993; Spagarino et al. 2001]. Skeletonisers and chewers comprise various lepidoptera larvae in the Geometridae (e.g. *Warreniana* spp.), Noctuidae and Saturniidae (e.g. *Ormiscodes* spp.). Pit feeders are mostly Curculionidae (Coleoptera), and sap suckers include aphids (Heteroptera: Aphidoidea, e.g. *Neuquenaphis* sp.), jumping plant lice (Psyllidae, *Notophorina* sp.) and scale insects (Coccoidea; e.g. Eriococcidae; McQuillan 1993; Spagarino et al. 2001). We thoroughly searched for folivores in each of the approximately 6,000 sampled leaves per site (400 leaves \times 15 trees) and expressed folivore abundance as the number of individuals on a 100-leaf basis. Damage produced by each feeding guild was measured in 150 fully expanded leaves per site (10 leaves per tree). For each tree (branch), we selected the first five fully expanded leaves (starting from the base of the shoot) from the two basal shoots of the sample branch. Damage was expressed both as the percentage of leaf area damaged (except for sap suckers, pit feeders, and gallers) and frequency of damaged leaves (measured for all insect guilds). We generally used

the frequency of damaged leaves because it is the most suitable parameter to compare levels of damage among different feeding guilds and because it should be less sensitive to variation in sampling date within a growing season. Nonetheless, damage frequency was strongly correlated with the amount of leaf area damaged across study sites (Pearson's $r > 0.80$ for all insect guilds).

For each sampling date, we determined several leaf traits, including tissue concentrations (%) of nitrogen (N), phosphorus (P), potassium (K), water and total phenolics. We also measured leaf size, toughness and specific leaf area (SLA). Each variable was measured on a sub-sample of 45 fully expanded, non-senescent, undamaged leaves per site. We selected the first three leaves (after sub-sampling for leaf damage; see previous paragraph) from the two basal shoots of each sample branch, and we then pooled leaves from all trees sampled within a site. Leaf N content was determined by semi-micro Kjeldahl digestion. Leaf P and K concentrations were measured after humid acid ($\text{HNO}_3/\text{HClO}_4$) digestions and were determined by inductively coupled plasma–atomic emission spectrophotometry. Leaf water content was determined as the difference between wet and dry leaf weights (after drying at 70°C for 72 h) and was expressed as the percentage of leaf wet weight. Total phenolics were determined using the method described in Folgarait and Davidson (1994), with concentrations expressed as milligrams of gallic acid per gram of leaf dry weight. Leaf toughness was measured with a penetrometer as the weight needed to punch a hole through the laminae using a 1.6-mm-diameter steel rod (expressed in g mm^{-2}). Leaf size (cm^2) was derived from the following equation: $\text{size} = -0.68 + 0.91 \times \text{length} + 0.28 \times \text{length}^2$, which was fitted by least squares regression ($r^2 = 0.94$) based on 2,084 *N. pumilio* leaves taken from a range of forest sites. SLA was expressed as square millimetres of leaf area per milligram of dry weight.

For each study site, we obtained the mean annual temperature, mean temperature of the warmest quarter (i.e. during the growing season) and mean temperature of the coldest quarter (i.e. during the winter season) from the Worldclim (version 1.4) database (Hijmans et al. 2005). We could not extract climatic data for Cerro La Mona due to poor spatial resolution, as the three sites on this mountain fall within the same pixel (1 \times 1 km) of bioclimatic data (ESM Fig. S1). Thus, climatic analyses included 15 instead of 18 sites.

Reciprocal transplant experiment

In November 1999, *N. pumilio* seedlings (max. height 5 cm) were collected from each of two elevations (1,100 and 1,540 m a.s.l.) at Cerro Chall Huaco. Field collection of seedlings was preferred because of the extremely low

germination rates of *N. pumilio* (Premoli 2004). Previous studies of these populations had shown significant genetic and ecological differentiation (Premoli 2003; Premoli and Brewer 2007). The plants were cultivated for 6 years in a common environment within a naturally lighted greenhouse at Laboratorio Ecotono, Universidad Nacional del Comahue, Bariloche, which is located at 876 m a.s.l and 15 km north of the Chall Huaco sites (Premoli et al. 2007). Seedlings were tagged and planted in individual pots filled with a commercial soil mixture. All plants were equally watered and fertilised, and their positions within the greenhouse were randomised and periodically rotated (for further details, see Premoli et al. 2007).

In May 2005, 45 plants from each population of different elevation origin were planted both in a low- and in a high-elevation site at Cerro Chall Huaco (180 plants in total). Within each site, plants of the two populations were randomly assigned for planting within a 1,000-m² area. During two growing seasons (November 2005 to March 2006 and November 2006 to March 2007), we measured leaf damage and insect folivore abundance on each transplant by means of non-destructive field censuses. Leaf damage was estimated for 30 leaves per plant by selecting approximately six leaves located near the base of each of five branches selected to represent different positions within the plant (all plants had fewer than 10 branches). Insects were counted by thoroughly searching the whole plant foliage. Census dates were 18–19 February 2006, 5–6 April 2006, 15 November 2006, 20–21 December 2006, 30–31 January 2007 and 28–29 March 2007. In November 2006, sampling was performed only at the low-elevation site because leaves had not yet flushed at the high-elevation site.

During the experimental period, temperature and relative humidity were measured daily at both study sites using HOBO H8 loggers (Onset Computer Corp, Bourne, MA). Mean annual temperature was 6.4°C and 5.7°C for the low- and high-elevation sites, respectively. The minimum temperature was –7.9°C for the low-elevation site and –11.7°C for the high-elevation site (winter 2006); the maximum temperature was 34.4°C for the low-elevation site and 34.9°C for the high-elevation site. Mean annual relative humidity was 77.8 and 64.7% for the low- and high-elevation sites, respectively. At both sites, mean temperatures and relative humidity were 0.2°C and 8.6% lower in 2006–2007, relative to 2005–2006.

Statistical analyses

For the regional sampling, we evaluated whether elevation affected insect abundance, leaf damage and leaf traits. Data were analysed through mixed-effect models, which tested for the fixed effects of elevation (low, middle and high),

date (January 2006, April 2006 and April 2007) and elevation × date interaction (both elevation and date were treated as categorical variables). Since the six study mountains were located along a strong west-to-east precipitation gradient, we added the mean annual precipitation (same value for the three sites along a mountain slope), precipitation × elevation and precipitation × date interactions as fixed-effect covariates to reduce the amount of unexplained variance. Random effects were evaluated through a random intercept model (Zuur et al. 2009), which considered different sampling dates as repeated measures within sites (three dates for each of 18 sites) and that sites were nested within mountains (three sites for each of six mountains; see ESM Tables S1, S2). Residuals of this model showed no correlation among dates for all response variables. Sequential analysis of variance (ANOVA) was performed on the full model for each response variable (Tables 1, 2). Insect abundance and leaf damage frequency by miners, chewers and gallers were square root transformed to meet ANOVA assumptions, although untransformed data are presented in the figures.

We used path analysis (Shipley 2000) to evaluate a conceptual model for the influence of elevation on the frequency of damaged leaves as mediated by changes in tree leaf traits and total insect abundance. We tested whether elevation affected insect abundance directly or indirectly via changes in leaf traits. In addition, we examined the direct associations of herbivore abundance and leaf traits with leaf damage frequency. This one-model approach allowed us to evaluate the extent to which leaf traits influenced herbivore damage through effects on insect abundances and/or insect feeding rates. The model included five variables for each forest site: elevation, leaf size, foliar P content, total insect abundance and total frequency of damaged leaves. Thus, the model yielded five variance parameters, one per variable, and eight effect parameters. The model estimation was based on standardised data through correlation coefficients. We chose leaf size and P concentration to characterise tree leaf traits because these showed strong elevational trends and were not significantly correlated to each other (see “Results”). Elevation was included as a quantitative variable (metres); a zero value was assigned to the low-elevation sites, and the mid- and high-elevation sites were then expressed as the difference in metres relative to the low-elevation site along the same mountain slope. For the plant and insect variables, we used average values for April 2006 and April 2007, which yielded 15 observations (5 mountains × 3 elevations, excluding Cerro Bayo; see “Regional sampling”). We did not include temperature in the path analysis due to its strong covariation with elevation (Pearson’s $r = -0.93$, $P < 0.0001$) and because climatic data were not available for Cerro La Mona. Still, we calculated

Table 1 ANOVA of changes in insect abundance and leaf damage by different feeding guilds across *Nothofagus pumilio* forests located at three contrasting positions along replicated elevational gradients

Variables	Total folivore abundance	Total area damage	Total damage frequency	Skeletonisers	Pit feeders	Miners	Chewers	Gallers	Suckers
Precipitation (1, 4) ^a	0.01	0.8	0.2	0.03	1.7	0.1	0.7	0.3	2.4
Elevation (2, 8)	16.5**	12.6**	31.2***	15.4**	13.8**	26.0***	2.0	17.6**	13.3**
Elevation × precipitation (2, 8)	0.9	1.5	0.2	1.6	0.7	0.5	0.9	1.7	2.6
Date (2, 24)	4.5*	16.2***	29.6***	13.3***	2.4	26.5***	34.1***	2.0	3.2*
Elevation × date (4, 24)	0.3	2.7	1.0	1.6	0.9	4.3**	0.7	1.4	0.4
Precipitation × date (2, 24)	4.0*	1.6	3.5*	2.0	1.6	1.9	0.9	0.4	0.3

Significant effects at: *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$

Each feeding guild was represented by the frequency of damaged leaves per plant (damage by other insect guilds was too low to warrant statistical analyses). Values show F statistics for each model term

ANOVA Analysis of variance

^a The degrees of freedom for the numerator and the denominator of F ratios are given in parenthesis, in that order

Table 2 ANOVA of changes in leaf traits across *N. pumilio* forests located at three contrasting positions along replicated elevational gradients

Variables	Leaf size (cm ²)	SLA (mm ² mg ⁻¹)	Toughness (g mm ⁻²)	N (%)	P (%)	K (%)	Phenol (mg gallic acid/g dw)	Water (%)
Precipitation	0.7	0.04	1.8	0.2	0.1	5.0	0.01	0.3
Elevation	98.9***	4.9*	0.5	0.4	4.9*	0.3	0.6	1.3
Elevation × precipitation	0.4	0.2	4.4	2.2	0.3	0.1	1.5	0.7
Date	0.4	0.8	8.5**	35.2***	4.0*	9.9***	17.5***	2.1
Elevation × date	0.4	0.2	4.7**	2.2	1.1	1.1	1.5	1.1
Precipitation × date	0.1	1.4	0.2	0.7	0.1	2.0	1.2	0.5

Significant effects at: *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$

Values show F statistics for each model term (degrees of freedom for F tests are the same as in Table 1)

SLA Specific leaf area, N nitrogen, P phosphorus, K potassium, dw dry weight

Pearson's correlations of mean temperature with leaf damage and insect abundance. Mean annual precipitation did not covary with elevation in this dataset (Pearson's $r = -0.43$, $P = 0.11$).

The reciprocal transplant experiment was analysed using mixed-effect models to test for the fixed effects of plant origin (low and high elevation), site (low and high elevation), year (2006 and 2007) and their interactions on leaf damage levels (see ESM Table S3). Data for November and December 2006 were excluded from these analyses because either the leaves had not yet flushed (November) or there was no leaf damage (December) in the upper site (data not shown). Leaf damage data were averaged within the first (February and April 2006) and second (January and March 2007) study seasons to ensure normality of error distributions. Data were square root transformed prior to analysis, although untransformed data are presented in figures. Random effects were evaluated through a random intercept model (Zuur et al. 2009), which considered that sampling dates were repeated measures of the same plants; residuals showed no correlation between years for any

response variable. Sequential ANOVA was performed on the full model for each response variable. Insect abundances were too low to warrant analysis for the first year of the experiment, and so analyses focussed on total folivore abundances in the second year. All statistical analyses were performed using R software (R Development Core Team 2009). Mixed-effect models for the regional sampling and the transplant experiment were fitted using the lme function of the nlme package (Pinheiro et al. 2009), whereas the path analysis was implemented using the sem package.

Results

Patterns along elevation gradients

Total insect abundance, leaf damage frequency and leaf area damage were highest at the lowest elevation site, generally decreasing from low- through mid- to high-elevation sites (Table 1, Fig. 1). Overall, folivore abundance was 14-fold higher in low-elevation sites than in high-

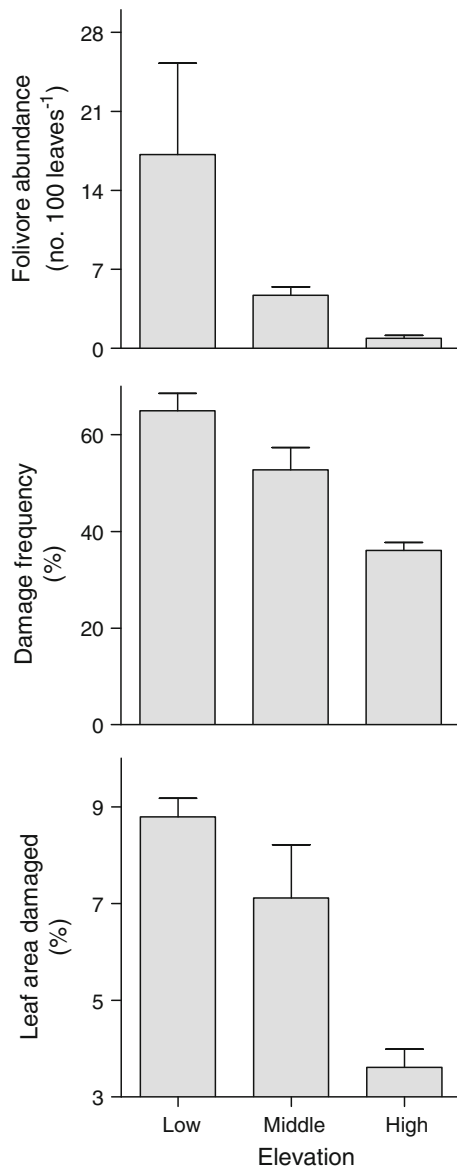


Fig. 1 Total abundance of insect folivores and leaf damage [mean \pm standard error (SE)] in *Nothofagus pumilio* forests located at three contrasting positions along replicated elevational gradients ($n = 6$ mountains). Data shown are average values for two growing seasons (April 2006 and 2007)

elevation sites, while leaf damage frequency and leaf area damage were twofold and 2.5-fold higher in low-elevation than in high-elevation sites, respectively (Fig. 1, ESM Table S1). Leaf damage increased from mid summer (January) to early fall (April) 2006; late-season damage was lower in April 2007 than in April 2006 (Table 1, ESM S1). Mean annual precipitation had a variable relationship with insect abundance and leaf damage frequency that depended on the sampling date (Table 1).

Leaf damage produced by different feeding guilds consistently decreased with increasing elevation (Table 1, Fig. 2). The effect of elevation on leaf damage was

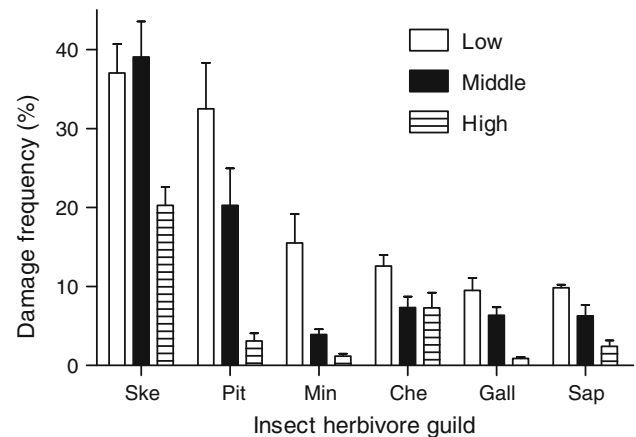


Fig. 2 Frequency of damaged leaves by different insect guilds (mean \pm SE) in *N. pumilio* forests located at three contrasting positions along replicated elevational gradients ($n = 6$ mountains). Data shown are average values for two growing seasons (April 2006 and 2007). *Ske* Skeletonisers, *Pit* pit feeders, *Min* miners, *Che* chewers, *Gall* gallers, *Sap* sap suckers

independent of sampling date, except for leaf miners, which showed greater differences in April 2006 and 2007 than in January 2006 (Table 1, ESM Table S1). Relative differences in leaf damage between high- and low-elevation sites (averaged for April 2006 and April 2007) were approximately 90% for pit feeders, miners and gallers, approximately 80% for leaf tiers and sap suckers and approximately 40% for skeletonisers and chewers (ESM Table S1). Mean annual precipitation did not covary with leaf damage levels from any insect guild, and elevational effects were independent of precipitation (Table 1).

Some of the measured leaf traits showed strong covariation with elevation (Table 2). Leaf size and SLA were 2.7- and 1.2-fold higher in low-elevation sites than in high-elevation sites, respectively, whereas leaf P content was 1.5-fold lower in the former compared to the latter (Fig. 3). Indeed, leaf size and SLA were positively correlated across sites (see ESM Table S4). Water, N, K and phenolic contents did not consistently vary with elevation, whereas leaf toughness showed variable patterns among sampling dates (Table 2, ESM Table S2, ESM Fig. S2).

The chi-square test, Bentler CFI and Bentler–Bonnett NFI all indicated that the path model had an adequate data fit (Fig. 4). Path analysis showed that both total folivore abundance and foliar traits were important in mediating the influence of elevation on the frequency of damaged leaves (Fig. 4). Elevation had a negative influence on leaf damage that was mediated by a decrease in folivore abundance. In addition, although there was no association between leaf traits (leaf size or P content) and insect abundance, elevation significantly affected leaf damage levels through its negative influence on leaf size. Leaf size and SLA were positively correlated with insect damage frequency,

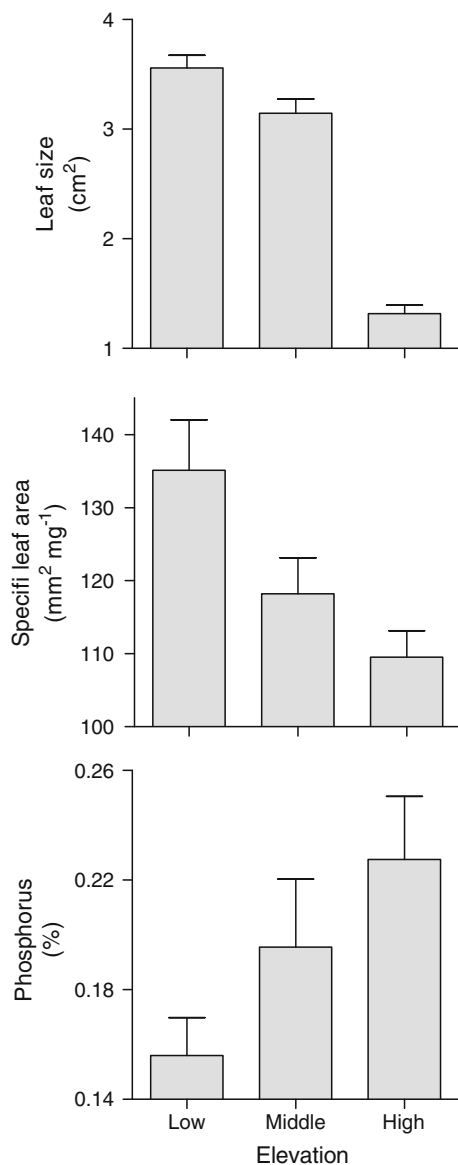


Fig. 3 Leaf size, specific leaf area and phosphorus content of *N. pumilio* trees located at three contrasting positions along replicated elevational gradients ($n = 6$ mountains). Data shown are average values for all dates

whereas P content was negatively correlated with leaf damage ($P < 0.05$). Leaf size showed the highest correlation coefficients across all three sampling dates (Pearson's r for leaf damage frequency: January 2006, $r = 0.73$, $P = 0.001$; April 2006, $r = 0.87$, $P < 0.001$; April 2007, $r = 0.63$, $P = 0.005$). Mean temperature was positively correlated with both total insect abundance and leaf damage measures across sites (see ESM Table S5).

Reciprocal transplant experiment

In *N. pumilio* transplants, total insect abundance, leaf damage frequency and leaf area damaged were all higher

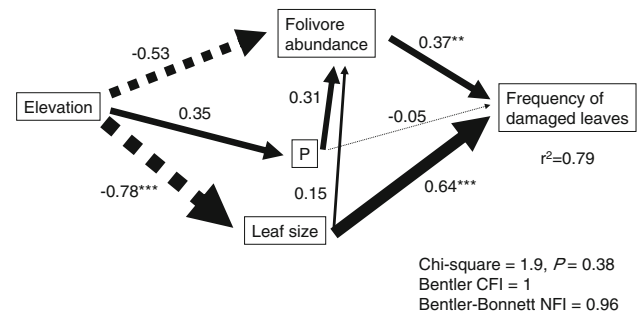


Fig. 4 Path analysis for the influence of elevation on the frequency of damaged leaves as mediated by changes in folivore abundance and leaf traits in *N. pumilio* forests. Arrow thickness indicates the standardised magnitude of each factor (from 0 to 1), asterisks denote significant factors: *** $P < 0.001$, ** $P < 0.01$. Dashed and solid lines refer to negative and positive relationships, respectively

for plants at the low-elevation site than for those at the high-elevation site (Table 3, Fig. 5, ESM Table S3). Leaf damage frequency, averaged between April 2006 and 2007, was 0.69 and 0.30 in the low- and high-elevation sites, respectively (Fig. 5). Furthermore, on average, *N. pumilio* plants originating from the low-elevation site experienced greater damage frequency and leaf area damage and also supported higher folivore loads than plants from the high-elevation site (Table 3, Fig. 5, ESM Table S3). Averaged between April 2006 and 2007, leaf damage frequency was 0.55 and 0.37 for plants of low- and high-elevation origin, respectively (Table 3, Fig. 5). Differences in leaf damage between saplings of contrasting origin were larger in the high-elevation site than in the low-elevation one (Table 3, Fig. 5). Overall, the difference in the frequency of damaged leaves between sites ($0.69 - 0.30 = 0.39$) was two-fold larger than the difference between origins ($0.55 - 0.37 = 0.18$).

Sapling leaf damage by all insect feeding guilds decreased with elevation in both study years. For the second year, elevational differences in herbivory increased for skeletonisers, pit feeders and leaf miners, but decreased for chewers (Table 3, Fig. 6). Plants originating from the high-elevation site were less damaged by skeletonisers and miners than those from the low-elevation site; for leaf miners, such differences occurred mostly within the lower forest site (Table 3, Fig. 6). Leaf chewer and pit feeder damage did not significantly differ between plants of contrasting origin, although pit feeder damage showed a similar pattern to that described for leaf miner damage (Table 3, Fig. 6).

Discussion

Environmental conditions may affect plant–insect herbivore interactions through changes in plant resistance to

Table 3 ANOVA of changes in insect abundance and leaf damage by different feeding guilds on *N. pumilio* saplings from low- and high-elevation forest sites (origin effect) planted at low- and high-elevation forests (site effect)

	Total folivore abundance	Total area damage	Total damage frequency	Skeletonisers	Pit feeders	Miners	Chewers
Origin	8.5**	3.1 [†]	9.9**	5.6**	1.5	1.1	0.3
Site	5.6*	93.8***	123.8***	6.7*	87.6***	154.4***	312.4***
Date	–	0.02	0.3	2.4	60.3***	1.2	4.9*
Origin × site	0.4	2.8 [†]	2.6 [†]	2.7 [†]	2.0	2.6 [†]	0.1
Origin × date	–	2.8 [†]	2.1	2.1	0.03	0.4	0.01
Site × date	–	14.7***	13.8***	6.2**	31.3***	9.3***	9.3***
Origin × site × date	–	0.3	0.5	0.02	0.1	0.3	1.8

Significant effects: *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$, [†] $P < 0.10$

Each feeding guild was represented by the frequency of damaged leaves per plant (damage by other insect guilds was too low to warrant statistical analyses). Values show *F* statistics for each model term. Degrees of freedom are 1 and 99 for the numerator and denominator of *F* ratios for all tests, respectively

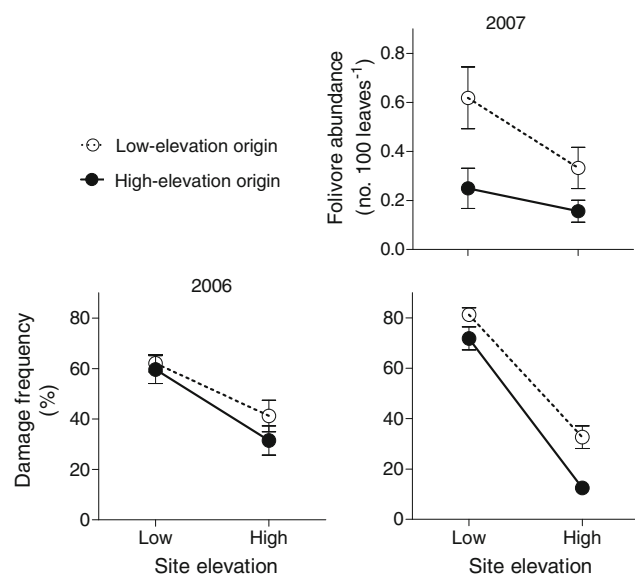


Fig. 5 Total abundance of insect folivores and leaf damage frequency (mean ± SE) in *N. pumilio* saplings planted in low- and high-elevation forest sites. Saplings were collected from low- (open symbols) and high- (solid symbols) elevation forest sites, grown in a common environment for 6 years and then taken back to the field sites. Folivore abundances are shown for the second year of the reciprocal transplant experiment

herbivory and/or insect population abundances (Hodkinson 2005). Accordingly, we found strong elevational variation in tree leaf traits and folivore insect loads, and both these factors were associated to a decrease in folivory by different insect guilds with increasing elevation. Furthermore, reciprocal transplants showed that tree genotypes from a low-elevation site had lower constitutive resistance to insect folivory and thus experienced higher damage than trees from a high-elevation site. Even so, our experiment suggested that local environmental conditions—rather than

host-tree genotype—exerted the predominant influence on herbivory along this forest elevational gradient.

Insect abundance decreased towards higher sites, which is in agreement with an herbivore-driven control of tree folivory along the elevational gradient. This trend was not influenced by observed shifts in leaf traits (Fig. 4), but likely reflected the direct impact on insect populations of decreased mean temperatures at high elevations (ESM Table S5). A cooler climate may reduce larval growth rates and extend developmental time, thus decreasing insect survival (Whittaker and Tribe 1996; Hodkinson 1997; Williams 1999). In accordance with this theory, a study in low-elevation *N. pumilio* forests showed that, during a 2-year spell of unusually dry and warm conditions, leaf damage by chewing insects increased above the mean levels recorded during 5 years of average climatic conditions (Mazía et al. 2009). Our results concur with those of prior studies indicating greater abiotic limitations to insect herbivores at higher elevations (Suzuki 1998; Alonso 1999; Bale et al. 2002). Conversely, other studies have suggested that effects of low temperature on herbivorous insects at higher elevations may be obscured by positive effects derived from reduced natural enemy pressure (Koptur 1985; Hodkinson 2005). While elevation is certainly a complex ecological gradient (Körner 2007), our results suggest that habitat constraints reduced the activity of insect folivores (at least) as much as that of their natural enemies. More studies are needed to elucidate the role of top-down control of insect herbivory along elevational gradients.

We found that leaf size and SLA of mature *N. pumilio* trees decreased with elevation. Likewise, Barrera et al. (2000) reported that *N. pumilio* trees had smaller leaves and reduced growth rates at high-elevation sites compared to low-elevation sites in Tierra del Fuego, southern Patagonia. Leaf size and SLA have also been associated with

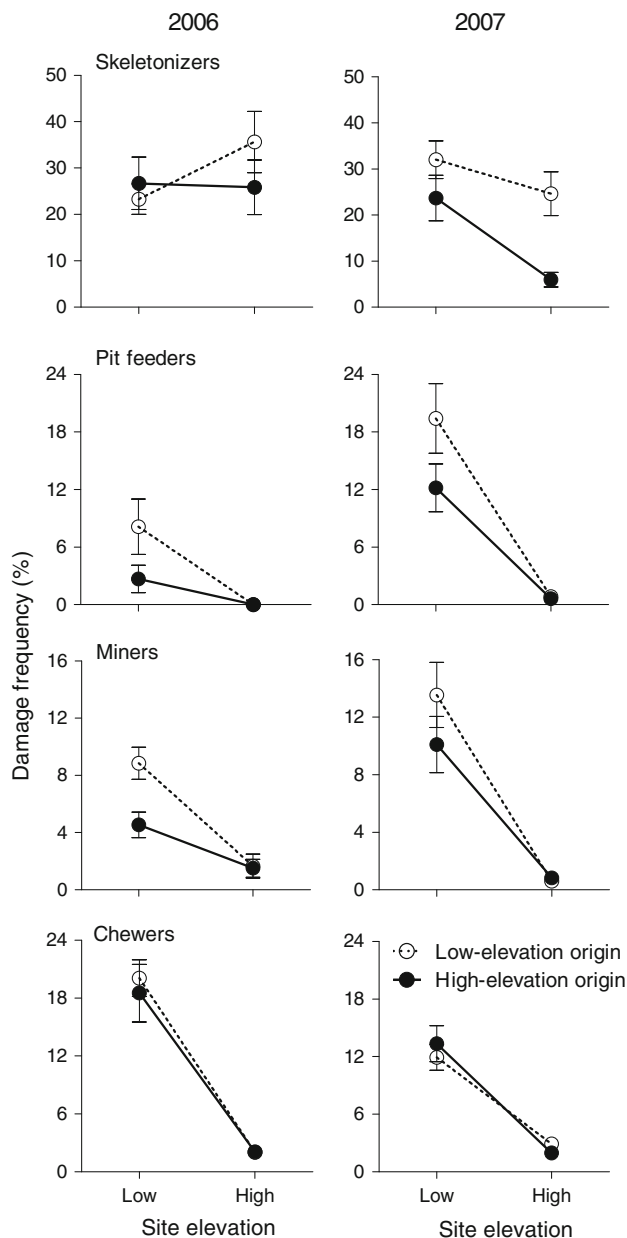


Fig. 6 Frequency of damaged leaves by different insect guilds (mean \pm SE) in *N. pumilio* saplings planted in low- and high-elevation forest sites. Saplings were collected from low- (open symbols) and high- (solid symbols) elevation forest sites, grown in a common environment for 6 years and then taken back to the field sites

tree growth in other forest ecosystems (Ackerly and Reich 1999). These changes in leaf traits may indicate that environmental conditions also became more limiting to trees in the upper mountain sites (Diemer 1996; Suzuki 1998). Low temperatures and partial CO_2 pressures, as well as a shorter growing season, may all constrain carbon uptake and plant growth at high elevations (Hodkinson 2005; Srur et al. 2008). In our study, elevational shifts in leaf traits were strongly correlated with insect folivory, a

pattern that is consistent with a bottom-up control of herbivory mediated by changes in tree physiology.

Although we did not aim to identify critical foliar traits, some variables suggested an elevational decline in leaf quality to insects. First, small leaves can reduce the foraging efficiency of insect folivores (Brown et al. 1991) and may be less vulnerable to herbivores due to slower expansion rates (Kursar and Coley 2003; Coley et al. 2006) and shorter duration of expansion (Coley and Barone 1996; Moles and Westoby 2000). Also, leaf size has been found to increase resource quality for mining and tying insects (Kagata and Ohgushi 2001; Marquis et al. 2002; Low et al. 2009). Second, *N. pumilio* trees at higher elevations possess higher leaf carbon, lignin and lignin/N contents than trees at lower elevations (Premoli 2004), which reinforces the idea that the former may offer less palatable foliage to insects. Thus, elevational variation in the abiotic environment would appear to control folivory through changes in foliar traits as well as changes in insect population abundances.

Leaf P concentration generally increased with elevation, which is consistent with global patterns of leaf nutritional quality (Körner 1989) and may reflect environmentally induced reductions in tree growth rates or higher nutrient supply rates (Chapin and Oechel 1983). Nevertheless, although plants at the high-elevation site had potentially higher nutritional quality, insect abundance and leaf damage decreased with elevation and were negatively correlated with leaf P levels. These results contrast with those of Erelli et al. (1998) who found that leaf nutrients had a preeminent role in determining the higher performance of herbivorous insects fed with tree foliage from high-elevation sites. Furthermore, our reciprocal transplants show that high-elevation trees supported consistently lower insect abundances and were less damaged than low-elevation trees, at both planting sites. While feeding assays using individual insects may shed light on relative herbivore preferences for different leaf materials (e.g. Erelli et al. 1998), their results may not be directly extrapolated to the total process of insect herbivory in field settings (Suzuki 1998).

Although the transplant experiment was carried out on a single mountain with only two populations, its results confirmed the regional patterns. Moreover, we found evidence suggesting increased constitutive resistance of high-elevation trees to insect folivory. Plant defence theory predicts that plants with low intrinsic growth rates should be better defended against herbivory than fast-growing plants (Herms and Mattson 1992; Stamp 2003). Indeed, trait adaptations to certain abiotic stressors may provide additional advantages in the presence of herbivores (Coughenour 1985; Coley 1987). Premoli et al. (2007) reported that *N. pumilio* plants of high-elevation origin

showed reduced shoot annual growth and leaf size, relative to plants of low-elevation origin, after 4 years in a common environment. These results, together those on our reciprocal transplants, suggest a trade-off between herbivory resistance and growth rate for *N. pumilio* populations occurring at different elevations. Saplings from the low-elevation site were more susceptible to folivore damage (this study) and showed higher growth rates (Premoli et al. 2007) than saplings from the high-elevation site. Additionally, Premoli et al. (2007) found a 2-week delay in leafing phenology and a shorter period of leaf exposure in high-elevation saplings compared to low-elevation saplings. Thus, elevational differences in folivory may also reflect a shorter window of plant susceptibility to insect attack and/or the asynchrony of insect development with genetically controlled leaf phenology (Aizen and Patterson 1995; Mopper and Simberloff 1995).

Few experiments have examined the relative roles of plant genetic differentiation and environmental variation in determining plant–herbivore interactions along habitat stress gradients (Fine et al. 2004; Brenes-Arguedas et al. 2009). In our study, genotypic resistance to insect damage appeared to be less important than local site factors in explaining folivory patterns. Average differences in leaf damage between the low- and high-elevation sites were twofold greater than the difference observed between plants from contrasting origins growing within a common site. We do not know at present the extent to which this environmental effect was mediated by changes in folivore abundance, phenotypic plasticity of certain traits (e.g. leaf size), or both. From a plant perspective, it is conceivable that both constitutive and plastic trait variation could have led to higher insect resistance and lower folivory in high-elevation forests.

Several different feeding guilds of endophagous and exophagous insects showed qualitatively similar responses to environmental conditions and host-plant genotype. These patterns were consistent with the notion that temperature at higher elevations is suboptimal for most feeding guilds (Deutsch et al. 2008). While we did find some guild-specific differences in the magnitude of response to elevation, observed patterns were not as expected (Sinclair and Hughes 2010). There were no consistent differences between internal (miners and galls) and external (skel-tonisers, chewers, pit feeders, sap suckers) insect feeders (Figs. 2, 6). These results suggest that the endogenous habit may not confer a definite advantage to folivores—compared to their exogenous counterparts—to cope with the increasingly harsher conditions typical of high-elevation environments. In addition, the fact that plants originating from high-elevation sites received the lowest damage from a range of different feeding guilds suggests a positive covariation of plant traits conferring resistance to different folivore guilds (Agrawal 2007).

Elevational gradients have been under-explored within the framework of the effects of climate change on plant–insect interactions (Hodkinson 2005; Tylianakis et al. 2008; Wilf 2008). Our findings agree with those of prior studies anticipating dramatic changes in insect herbivory with environmental warming (Bale et al. 2002; Currano et al. 2008; Wilf 2008). Further, they are broadly consistent with the increase in insect herbivore performance reported by those studies in which temperature was raised experimentally (reviewed by Zvereva and Kozlov 2006). Over geological time scales, the leaf fossil record shows higher insect leaf damage during the Paleocene–Eocene Thermal Maximum (55.8 Ma), when an abrupt warming episode took place (Currano et al. 2008 and references therein). Current ensembles of general circulation models (SRES AR4 A2 experiments) predict for northern Patagonia a median rise of 1.5 and 2.7°C in mean annual temperatures for the 2050 and 2080 decennia, respectively (Christensen et al. 2007; Parry et al. 2007). In our experiment, we estimate an increase in mean annual temperature from the upper to the lower sites of approximately 2.2°C (based on a 0.5°C 100 m⁻¹ lapse rate). This thermal gradient was associated with an approximately 50% increase in damage frequency by folivorous insects. Nevertheless, high-elevation genotypes placed in a low-elevation site experienced a lower (approx. 39%) increase in insect damage. Our results therefore suggest that insect herbivory would increase within this century in low-elevation *N. pumilio* forests, possibly accelerating the mortality of trees expected from the increased influence of other processes related to climate change, such as drought (Srur et al. 2008) and fire (Kitzberger et al. 2005; Mermoz et al. 2005). In contrast, at high-elevation forests, the potential short-term increase in folivory may be smaller than expected only from changes in insect abundance, since insects may confront more resistant tree genotypes. Therefore, we emphasise the need to consider the responses of both parties in predicting climate change effects on plant–insect herbivore interactions along environmental gradients.

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