Gregarious bamboo flowering opens a window of opportunity for regeneration in a temperate forest of Patagonia

Carla V. Giordano, Rodolfo A. Sánchez and Amy T. Austin

Instituto de Investigaciones Fisiológicas y Ecológicas Vinculadas a la Agricultura (IFEVA) and Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Facultad de Agronomía, Universidad de Buenos Aires, Av. San Martín 4453, Buenos Aires (C1417DSE), Argentina

Author for correspondence:
Amy T. Austin
Tel: +54 11 4524 8070
Fax: +54 11 4514 8730
Email: austin@agro.uba.ar

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Summary

- Rare gregarious flowering of understorey bamboo species occurs in temperate and subtropical forests around the world, but the ecological consequences of this phenomenon for forest regeneration are not well understood.
- Field experiments were conducted in an old-growth temperate forest in Patagonia, Argentina after a massive bamboo flowering event, to examine whether light quality and other changes in microhabitats could affect seed germination and growth of overstorey species.
- Germination of southern beech (*Nothofagus obliqua*) was positively correlated with red:far red (R:FR) ratios in a range of microhabitats generated by the death of the understorey bamboo (*Chusquea culeou*). Experimental modification of understorey R:FR ratios to mimic alternative light environments reversed this germination response in plots with senescent understorey, but not in plots with live bamboo. Laboratory incubations demonstrated a significant interaction between R:FR ratios and thermal amplitude in promoting seed germination. Microhabitats also significantly affected the growth of emerged seedlings.
- Microenvironmental changes generated by this flowering event appear to have opened a window of opportunity for germination and growth of overstorey species. We demonstrate that natural gradients in light quality associated with this ecological phenomenon are a major component affecting forest regeneration in this ecosystem.

Introduction

Bamboo grasses occupy a dominant position in the understorey in temperate and subtropical forests around the world. Many bamboo species have a peculiar life cycle, with long vegetative periods followed by synchronized flowering and senescence of the entire population over extensive areas, at intervals ranging from 6 to 120 yr (Janzen, 1976). The signaling mechanism for gregarious flowering of bamboo species is presently unknown but the phenomenon of massive flowering occurs in many parts of the world (Janzen, 1976; Keeley & Bond, 1999). The synchronized die-back of bamboos in the understorey has been linked to demographic explosions of granivores and threats to key specialist herbivores (Jaksic & Lima, 2003; Linderman *et al.*, 2005; Kitzberger *et al.*, 2007), and is thought to impact forest dynamics in South America (Veblen, 1982; Pearson *et al.*, 1994), Asia (Nakashizuka, 1988; Taylor *et al.*, 1995; Abe *et al.*, 2001) and Australia (Franklin, 2004). While there are frequently observed pulses of recruitment of overstorey vegetation that occur after bamboo flowering events (Abe *et al.*, 2001; González *et al.*, 2002; Holz & Veblen, 2006), the mechanisms that determine these episodes of regeneration are not clear.

There are a number of environmental cues that can affect seed germination in natural forest environments, including fluctuations in light quality (Grime *et al.*, 1981; Vázquez-Yanes & Smith, 1982; Pearson *et al.*, 2003), temperature (Vázquez-Yanes & Orozoco-Segovia, 1982; Thompson & Grime, 1983; Pearson *et al.*, 2002) and soil nutrient conditions (Bell *et al.*, 1999; Daws *et al.*, 2002). In addition, it appears that seed size can influence the light or temperature response of understorey and pioneer forest species. Studies have demonstrated that small-seeded species are more responsive to light quality (Jankowska-Blaszcuk & Daws, 2007) and large-seeded species to temperature fluctuations (Pearson *et al.*, 2002).

Changes in understorey conditions that can promote germination of overstorey species are often associated with the generation of canopy gaps (Vazquez-Yanes & Orozco-Segovia, 1993; Gray & Spies, 1997). In particular, the relative amounts of red (R; 600–700 nm) and far-red (FR; 700–800 nm) radiation change with variation in tree canopy or understorey cover. In forests, the interception of sunlight by a canopy of green vegetation reduces irradiance, but also changes its spectral composition, reducing the R:FR ratio as a result of preferential absorbance of red light (Holmes & Smith, 1975). Plant-phytochrome receptors perceive variations in R:FR ratios, which can elicit a series of plant responses including seed germination (Baskin & Baskin, 1998; Casal & Sánchez, 1998; Jankowska-Blaszcuk & Grubb, 2006) and the 'shade avoidance syndrome' whereby plants elongate and adjust their morphology and thereby avoid present and future light competition from neighbours (Morgan & Smith, 1976; Ballaré *et al.*, 1990).

It has been suggested that natural variation in light quality can modulate physiological plant responses with consequences at the individual, community and ecosystem levels (Smith, 1995; Ballaré, 1999) as a result of phytochrome perception of these gradients (Holmes & Smith, 1975; Morgan & Smith, 1976; Ballaré *et al.*, 1990; Vázquez-Yanes & Orozco-Segovia, 1994). Direct evidence of the photoblastic response of seed germination under natural conditions has been obtained in agroecosystems (e.g. Scopel *et al.*, 1991; Deregibus *et al.*, 2004). However, studies of germination responses of overstorey forest species to light quality in forests are relatively rare (Vázquez-Yanes & Smith, 1982; Vázquez-Yanes *et al.*, 1990; Caccia & Ballaré, 1998; Kyereh *et al.*, 1999; Pearson *et al.*, 2003), and few of these studies were conducted in field conditions. Moreover, these studies demonstrated a variety of species-specific responses to light quality that ranged from stimulation of germination, or an absence of a response to light quality, to a total dependence on fluctuating temperatures.

In the Southern Hemisphere summer of 2001, northwestern Patagonian temperate forests in Argentina experienced massive flowering of the native bamboo species *Chusquea culeou* (caña colihue), which forms a dense monospecific understorey in native southern beech forests of the region (Pearson *et al.*, 1994). The flowering and consequent senescence of the understorey extended over 200 000 ha; the previous documented bamboo flowering in the zone occurred in 1938 (Sanguinetti & García, 2001). This infrequent event represented a unique opportunity to study the influence of understorey bamboo flowering on the germination and growth of overstorey tree species. We hypothesized that the massive bamboo flowering event could elicit microenvironmental changes of sufficient magnitude to trigger substantial promotion of seed germination and seedling growth of the overstorey *Nothofagus* species. Further, we wanted to explore whether alterations of the light quality environment alone were sufficient to significantly alter rates of seed germination of overstorey species in this forest. We therefore characterized the natural light environment in the understorey in different forest microhabitats associated with the bamboo flowering and evaluated seedling emergence and growth of a dominant overstorey tree species, *Nothofagus obliqua*. In addition, we evaluated the sensitivity of this species to light quality and temperature fluctuations under laboratory conditions in an attempt to disentangle the multiple environmental changes that could occur as a result of this flowering event. The challenges of confounding ecological factors necessitated a manipulative approach in the field and laboratory, whereby many of these factors, such as seed dispersal and production, and predation pressure, could be eliminated. In this paper, we demonstrate that environmental changes resulting from massive bamboo flowering in the understorey play an important role in stimulating the germination and establishment of the overstorey species in these southern temperate forests.

Materials and Methods

Study site and microhabitat selection

The study site is an old-growth temperate forest in the Argentinean Andes (40°S 71°W) within the boundaries of Parque Nacional Lanín, at the northern side of Lake Lacar, 30 km west of the city of San Martín de los Andes. The site is located at an elevation of 800 m asl, with mean annual precipitation of 2300 mm, and mean minimum and maximum temperatures of 3°C (July) and 18°C (January). The forest canopy is co-dominated by three *Nothofagus* species: the deciduous *Nothofagus obliqua* (Mirb.) Blume and *Nothofagus nervosa* (Phil.) Dim. *et* Mil., and the evergreen *Nothofagus dombeyi* (Mirb.) Blume (Vivanco & Austin, 2008). The understorey vegetation, entirely dominated by the bamboo *Chusquea culeou* Desvaux, is dense and monospecific, with average aboveground biomass of approx. 3.5 kg m^{-2} (Marchesini *et al.*, in press). In this region, *C. culeou* covering an expanse of over 200 000 ha synchronously flowered and senesced in 2001, resulting in the death of 95% of the aboveground bamboo biomass (Sanguinetti & García, 2001; Marchesini *et al.*, in press), although small remnant patches of nonflowered green bamboo remained.

The study area within the forest site encompassed an area of approx. 50 ha on a south-facing slope. We installed plots in a range of forest microhabitats with the following characteristics (*n* = 10 in each microhabitat, with a total of 40 plots): forest with live bamboo understorey (FL); forest with senesced bamboo understorey (FS); canopy gaps with senesced bamboo (GS); and gaps where senesced bamboo culms were manually removed (GR). Ten naturally generated gap areas created by fallen trees, which were distributed throughout the study site (distance between gaps areas > 25 m), were identified. Adjacent to each of the gap areas, the associated microhabitats (FL, FS and GR) were located as close together as possible, such that the four associated microhabitats could be considered a block for statistical analysis.

Field seed germination experiments

Nothofagus obliqua seeds were collected in March–April of 2003 from a natural stand located near the study site in Parque Nacional Lanín, and stored in dry conditions at 6°C (Seeds Laboratory, INTA, Bariloche, Argentina). A subsample of the seeds (three subsets of 50 seeds) was tested for viability using a standard tetrazolium test, with 25% average viability determined for this seed batch. In early winter (June 2004), we installed 50 × 50 cm plots in the microhabitats described in the previous section. In each plot ($n = 10$ in each microhabitat), we removed fallen litter and sowed 225 *N. obliqua* seeds per plot; 150 three-winged and 75 two-winged achenes (2 : 1, the same proportion as found in seed capsules). Overwintering of the seeds in the soil maximized the possibility of alleviating innate seed dormancy (Baskin & Baskin, 1998; Casal & Sánchez, 1998). The seeds were surrounded and topped by wire mesh for protection from granivores (1.3 × 1.3 cm mesh size; 90% photosynthetically active radiation (PAR) transmittance). At the beginning of the growing season (early October) and at each subsequent sampling date, we counted the number of seedlings that had emerged from the experimentally sown seeds and assessed the survivorship of seedlings identified on previous sampling dates. The percentage of emerged seedlings was calculated as: (emerged seedlings/number of viable seeds sown in the plots) × 100. At the end of the growing season (March), we measured seedling height, number of leaves and internode number and length of surviving seedlings in each plot. During the course of the experiment, four plots were excluded from the analysis as a consequence of disturbance by animals and fallen branches. R:FR ratios at the soil surface in the forest microhabitats were measured near midday (11:00–14:00 h) at all sampling dates (October 2004–March 2005) using a 660/730-nm quantum sensor (Skye Instruments Ltd, Powys, UK). PAR was measured on later sampling dates (March 2005–January 2006) with a 400–700-nm line quantum sensor (Li 191 SA; Li-Cor Inc., Lincoln, NE, USA).

Manipulation of light quality in forest microhabitats

To directly control the light quality environment in the microhabitats, we additionally installed a manipulative experiment using plastic filters that attenuated R or FR light reaching the soil surface. The objective was to 'reverse' the original light quality environment using different plastic filters such that forest and gap microhabitats with senescent understorey mimicked the light environment of live green understorey (low R:FR ratios), while live understorey forest microhabitats mimicked the light environment of senescent understorey microhabitats (high R:FR ratios). Plots of identical size (50 × 50 cm) to those described in the previous section for the seed germination experiments were installed in three of the microhabitats: forest with live understorey (FL); forest with senescent understorey (FS); and gap with senescent understorey (GS). In the selected microhabitats, a paired neutral filter that did not alter the R:FR ratio but reduced total irradiance in equal proportion to the treatment filter was placed beside the treatment filters, and the area under this filter served as the control plot in each microhabitat ($n = 10$ for control and treatment in each microhabitat; total of 60 plots). For the controls in senesced understorey microhabitats (GS and FS), we used 3-mm-thick transparent acrylic sheets (Paolini SAIC, Buenos Aires, Argentina) covered with black nylon mesh that blocked 80% of incident PAR. For senescent understorey (FS and GS) −R plots (live understorey simulation), we used 3-mm-thick blue acrylic sheets (#2031; Paolini SAIC, Buenos Aires, Argentina) that filtered out ~95% of R quanta (80% PAR reduction) with little reduction in FR irradiance. At the same time, for controls in green understorey plots (FL), we used two-wall polycarbonate sheets filled with a transparent aqueous NaCl solution (7 g l^{-1}) covered with a black nylon mesh that blocked 20% of total radiation without altering its spectral distribution. For FL −FR plots (senescent understorey simulation), we used two-wall polycarbonate sheets filled with a $CuSO_4$ solution (90 g l^{-1}), which greatly reduced far-red transmittance with little effect on visible wavelengths (20% PAR reduction) (Ballaré *et al.*, 1991; Insausti *et al.*, 1995). Filters were placed 20 cm above the soil surface on wire mesh cages, with the south side of the plots left uncovered to enhance air circulation. The air temperature of representative experimental plots was measured hourly with HOBO® data loggers (Onset, Bourne, MA, USA) inside one pair of control and −R plots in an FS microhabitat and one pair of control and −FR plots in an FL microhabitat. R:FR ratios at the soil surface were measured with a 660/730-nm quantum sensor (Skye Instruments Ltd). Emergence of experimentally sown seeds was measured in an identical fashion as described for the field seed germination experiments.

Laboratory seed germination experiments

In an effort to disentangle the relative effects of changes in light quality and fluctuating temperature on *N. obliqua* seedling germination, we designed a factorial laboratory experiment with variation in temperature regime and light quality as the two main treatment factors. *Nothofagus obliqua* seeds were collected in 2007 from a natural stand located near the study site in Parque Nacional Lanín by personnel of INTA-Bariloche, and this collection was used for the laboratory germination experiments. A subsample of the seeds (three subsets of 50 seeds) was tested for viability using a

Table 1 Mean (SE) red:far-red (R:FR) ratios at the soil surface during the growing season 2004–2005 in different forest microhabitats: forest with live bamboo understorey (FL); forest with senesced bamboo understorey (FS); canopy gaps with senesced bamboo (GS); and gaps where senesced bamboo culms were manually removed (GR)

Midday R:FR ratio					
Microhabitat	Sep	Oct	Dec	Jan	Season
FL	0.69 (0.022) ^{a}	0.48 (0.041) ^{a}	0.39 (0.046) ^{a}	0.32 (0.037) ^{a}	0.44 (0.038) ^{a}
FS	1.01 (0.021) ^{b}	0.73 (0.023) ^{b}	0.72 (0.032) ^{b}	0.71 (0.035) ^{b}	0.73 (0.022) ^{b}
GS	1.11 (0.009) ^{c}	0.97 (0.038) ^{c}	0.89 (0.036) ^{c}	0.92 (0.045) ^{b}	0.92 (0.013) ^{c}
GR	1.13 (0.005) ^{c}	1.03 (0.018) ^{c}	0.97 (0.038) ^{c}	0.95 (0.041) ^{b}	1.00 (0.025) ^{c}
P-value	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001

P-values from one-way ANOVA are shown. Different superscript letters indicate significant differences in the same column according to a post-hoc Tukey Honest Significant Difference (HSD) test.

standard tetrazolium test, with a 70% average viability determined for this seed batch, and average seed mass = 18.0 (± 1.3) mg. For the germination experiment, 600 intact seeds were selected (half three-winged achenes and half two-winged achenes) and 30 seeds were placed in each of 20 plastic boxes (42 × 35 × 20 mm) with cotton batting. Distilled water was added to the boxes until seeds and cotton batting were completely moistened. Seed boxes were covered in 3-mm-thick black plastic film and were cold-stratified (4°C) for 8 wk before the initiation of the experiment to mimic natural overwintering conditions and in order to break embryo dormancy (Gunn, 2006). The temperature and light treatments were as follows: FR and constant temperature; FR and alternating temperature; R and constant temperature; and R and alternating temperature. There were five replicate boxes per treatment. At the initiation of the experiment, all seeds were exposed to a 20-min FR pulse (15 μmol m^{-2} s^{-1}) of light from 125-W incandescent internal reflector lamps filtered through an RG9 Schott glass filter (Schott AG, Mainz, Germany) and a 10-cm water filter. Half of the boxes (R treatment) were then exposed to a single 30-min red light pulse from Philips 40/15 40-W fluorescent lamps (Philips, Eindhoven, the Netherlands). After the light treatments, the seed boxes were again wrapped in black plastic and incubated in darkness in growth chambers at constant (20°C) or alternating (12 h at 15°C and 12 h at 25°C) temperatures. Seed germination was evaluated every 3 d for a 2-wk period following the initiation of the treatments. The percentage of germination was calculated as: (number of germinated seeds/number of viable seeds sown per box) × 100.

Statistical analysis

R:FR ratios, seedling emergence in the field and tree growth variables (tree height, number of leaves, and internode length) were evaluated using a one-way ANOVA (with microhabitat as the main factor) and simple linear regression of germination and R:FR ratios. Germination in the laboratory experiment was analyzed with a two-way ANOVA, with red light pulses and temperature as the two main factors. All data for seed germination (%) were arc-sine transformed before analysis. Normality was tested with the Shapiro–Wilks statistic and homoscedasticity with a Levene's test. In the case of tree seedling height data, values were transformed using the equation $x' = 1/x$ to correct for homogeneity of variance. Post-hoc comparisons were performed with a Tukey Honest Significant Difference (HSD) test for field and laboratory experiments on seedling germination; an α of 0.05 was used for all statistical analyses.

Results

The massive flowering event of the understorey created a gradient of light conditions, which included alteration of midday R:FR ratios that were associated with different degrees of bamboo senescence and overstorey canopy cover (Table 1, $P < 0.001$). The lowest R:FR ratios were consistently observed in the FL microhabitats, while the highest ratios were observed in the gap microhabitats (GS and GR) throughout the growing season. The presence of senesced bamboo versus the total absence of the understorey in the gaps did not affect R:FR ratios.

In *N. obliqua*, germination of experimentally sown seeds, which began in mid-October and lasted until early December, differed significantly among microhabitats (Fig. 1a, $P = 0.018$). The highest germination was observed in the gap microhabitats (GS and GR) and significantly lower germination occurred in the FL microhabitats (Fig. 1a). When considered as a light quality gradient, this pattern of germination was positively correlated with midday R:FR ratios (Fig. 1b; % germination = 40.18 × (R:FR) + 1.31, $r^2 = 0.32$, $P = 0.004$).

Installation of filters to simulate an alternative light quality environment in the microhabitats significantly altered R:FR ratios (Table 2); neutral filters that reduced overall irradiance did not affect light quality conditions (Tables 1, 2). In the FL microhabitat, –FR plots (senescent understorey simulation) had significantly increased R:FR ratios at all sampling dates

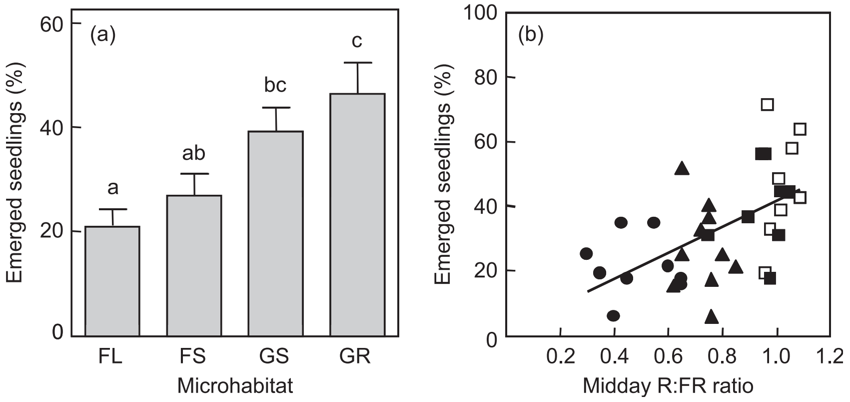


Fig. 1 (a) *Nothofagus obliqua* seedling emergence (% of viable sown seeds) in different forest microhabitats in a temperate Patagonian forest (abbreviations as defined in Table 1). Bars are means (+ SE); different letters indicate significant differences according to the post-hoc Tukey Honest Significant Difference (HSD) test. (b) Seedling emergence in microhabitats as a function of midday red:far-red (R:FR) ratios (Table 1); symbols indicate replicates of different microhabitats: forest with live understorey (FL, circles); forest with senescent understorey (FS, triangles); gap with senescent understorey (GS, closed squares); and gap with removed understorey (GR, open squares).

Table 2 Mean (SE) red:far-red (R:FR) ratios at the soil surface beneath plastic filters used in field manipulation experiments

Midday R:FR					
Microsite	Treatment	Sep	Oct	Dec	Season
FL	Control	0.72 (0.021)	0.53 (0.035)	0.41 (0.053)	0.47 (0.036)
	–FR	1.79 (0.069)	1.07 (0.09)	0.87 (0.089)	0.97 (0.072)
	P	< 0.0001	< 0.0001	< 0.0001	< 0.0001
FS	Control	1.01 (0.018)	0.74 (0.018)	0.70 (0.028)	0.72 (0.024)
	–R	0.003 (0.001)	0.007 (0.002)	0.004 (0.001)	0.006 (0.001)
	P	< 0.0001	< 0.0001	< 0.0001	< 0.0001
GS	Control	1.09 (0.012)	0.99 (0.034)	0.89 (0.031)	0.95 (0.024)
	–R	0.003 (0.001)	0.002 (0.001)	0.005 (0.001)	0.004 (0.001)
	P	< 0.0001	< 0.0001	< 0.0001	< 0.0001

The R:FR ratio was measured near midday at each sampling date.
FL, forest with live bamboo understorey; FS, forest with senesced bamboo understorey; GS, canopy gaps with senesced bamboo; GR, gaps where senesced bamboo culms were manually removed.

($P < 0.0001$; Table 2), with an average increase from 0.47 in the FL control plots to 0.97 in the –FR plots. This alteration in the FL microhabitats mimicked the conditions of an open gap microhabitat for light quality (high R:FR ratios), a technique that has been successfully used in other studies of light quality manipulation in the field (Ballaré *et al*., 1990; Insausti *et al*., 1995). Similarly, the –R filters placed in the FS and GS microhabitats (live understorey simulation) significantly reduced R:FR ratios (Table 2; $P < 0.0001$) at all sampling dates, although the actual R:FR ratios of the simulated dense canopy were below those naturally observed in FL microhabitats (Table 2). Thermal amplitude in neutral control and treatment filters did not differ within the same microhabitat (Fig. 2). However, there was a notable difference in thermal amplitude between microhabitats, with thermal amplitude being 2–5°C higher in plots of the FS microhabitats than in those of FL microhabitats, particularly in the early spring (Fig. 2).

The percentage of emerged seedlings in FS and GS microhabitats was significantly reduced when the spectral composition of the light environment mimicked a dense green canopy (Fig. 3a,b; $P = 0.028$ and 0.022 in GS and FS microhabitats, respectively). By contrast, alteration of the FL microhabitat to mimic the R:FR ratio of an open canopy did not significantly affect *N. obliqua* germination (Fig. 3c).

Laboratory incubations confirmed the effect of light quality on seed germination of *N. obliqua* and revealed a significant influence of alternating temperatures on the response to red light. Red light pulses significantly increased seed germination, but the magnitude of the response was determined by the interaction with alternating temperatures (Fig. 4; $P < 0.05$). The combination of red light pulses and alternating temperature showed highly stimulated germination (five times greater than that in control conditions of FR with constant temperature). Alternating temperatures alone were not sufficient to elicit changes in germination, with similar germination responses for the two temperature regimes (Fig. 4).

At the end of the first growing season, there were significant effects of microhabitat on the growth parameters of the

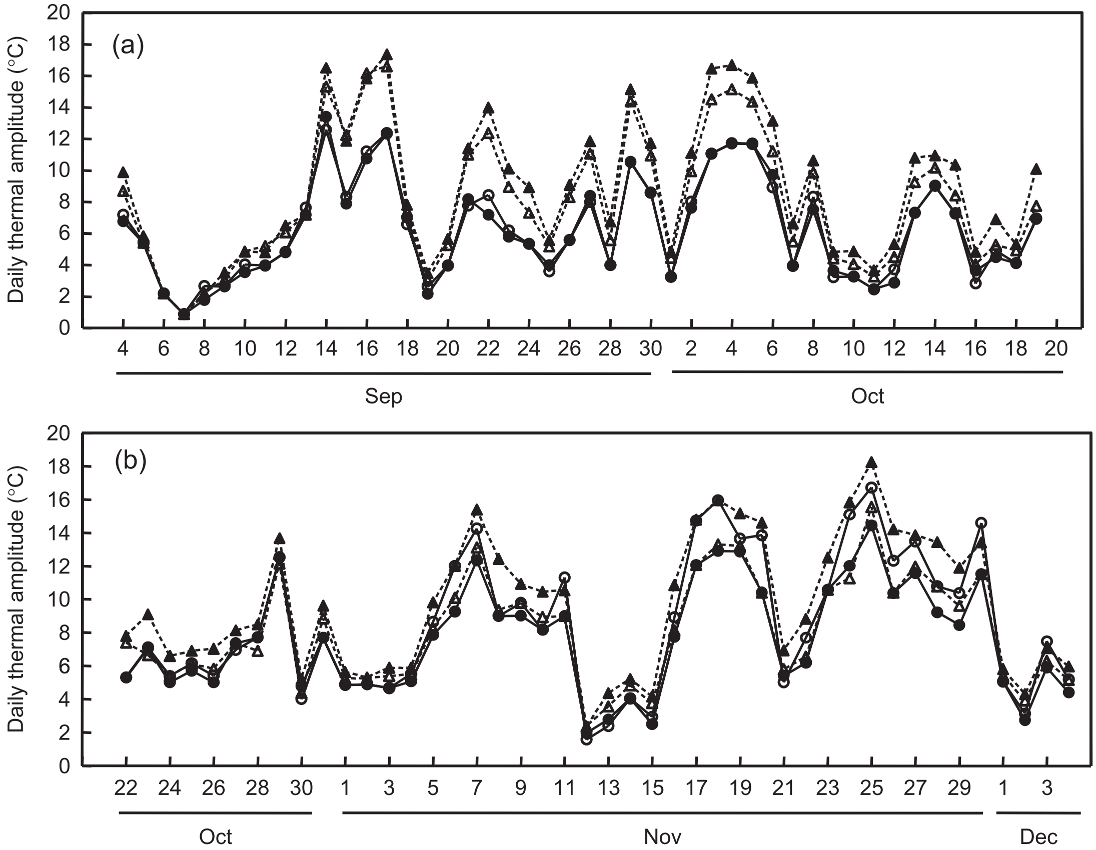
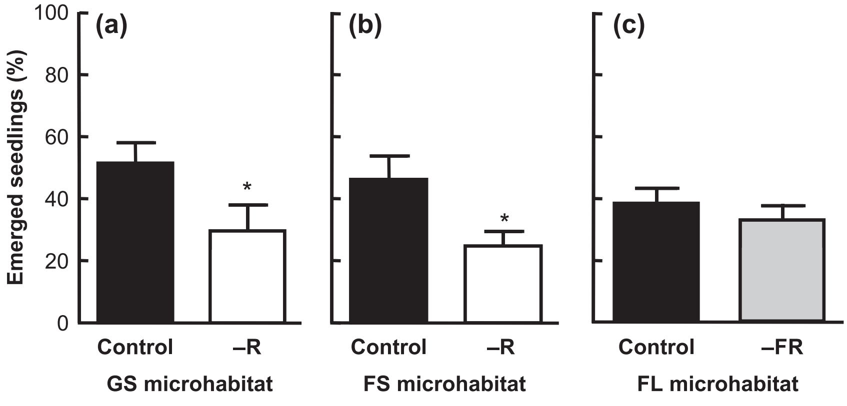


Fig. 2 Daily thermal amplitude in filter-covered plots in live understorey (FL) microhabitats: control (open circles) and minus far-red (–FR) plots (closed circles; senescent understorey simulation); and senesced understorey (FS) microhabitats: control (open triangles) and minus red (–R) plots (closed triangles; live understorey simulation). (a) September–October 2004; (b) October–December 2004.

Fig. 3 *Nothofagus obliqua* seedling emergence in paired plots with transparent plastic filters (control) and altered red:far-red (R:FR) ratios (–R, simulated understorey; –FR, simulated gap) in forest microhabitats (abbreviations as defined in Table 1). See Table 2 for R:FR ratios measured beneath the plastic filters. (a) Gap with senescent understorey (GS); (b) forest with senescent understorey (FS); (c) forest with live understorey (FL). Bars are means (+SE). Asterisks indicate significant differences between treatments ($P < 0.05$).



emerged seedlings under natural light conditions. These microhabitats differed not only in R:FR ratios, but also in total PAR irradiance (Table 3; $P < 0.0001$). Seedlings from microhabitats of live bamboo understorey (FL) had significantly longer internodes than seedlings grown in senesced microhabitats (FS, GS and GR) (Table 3; $P < 0.0001$). By contrast, seedlings in the gap sites (GS and GR) had more leaves than tree seedlings in forest microhabitats (Table 3; $P < 0.0001$). There was no effect on seedling height in spite of the significant differences in internode length and leaf production (Table 3; $P = 0.28$).

Discussion

We show that environmental changes brought about by a massive bamboo flowering event in this temperate Patagonian

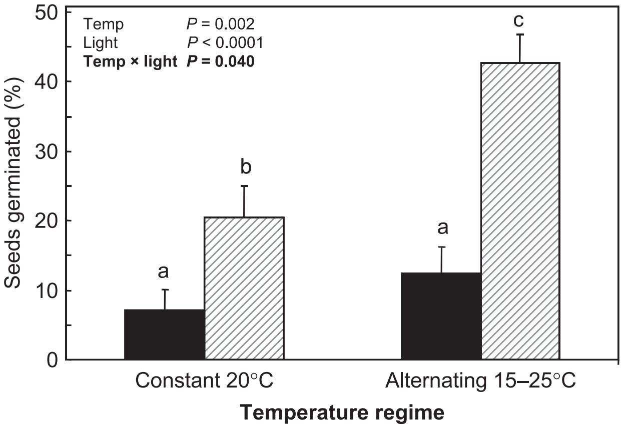


Fig. 4 *Nothofagus obliqua* seedling germination in laboratory incubations. Bars represent mean seedling germination (+SE; $n = 5$ per treatment) as per cent germination of viable seeds in the far-red (FR) treatment (closed bars) and the red (R) pulse treatment (hatched bars). Different letters indicate significant differences for post-hoc comparisons according to a post-hoc Tukey Honest Significant Difference (HSD) test.

forest had important consequences for seed germination and growth of *N. obliqua*, with implications for regeneration in these southern temperate forests. Ecophysiological studies on the consequences of bamboo flowering for overstorey regeneration up to now have focused on the liberation of resources, particularly light resources, to explain enhanced tree seedling recruitment and growth (Veblen, 1982; González *et al.*, 2002; Holz & Veblen, 2006; Taylor *et al.*, 2006). In addition, variation in total irradiance in forests with bamboo-dominant understoreys has been postulated as a mechanism to explain the co-existence of overstorey tree species (Taylor *et al.*, 2004). This study provides important information regarding a new axis of control, with respect to changes in light quality and temperature, of seed germination and seedling growth as a result of environmental changes naturally generated by bamboo flowering events.

The massive *C. culeou* flowering and senescence event in this temperate forest generated a gradient of R:FR ratios in the understorey (Table 1) that was sufficiently broad to suggest that a germination response would occur, based on laboratory and field studies in other species from tropical forests (Vázquez-Yanes & Smith, 1982; Vázquez-Yanes *et al.*, 1990), given that *N. obliqua* demonstrated sensitivity to light quality. The positive correlation of R:FR ratios and *N. obliqua* germination (Fig. 1b) suggests a phytochrome-mediated response to seed germination, and supports our original hypothesis that variation in light quality could alter seed germination in this ecosystem after a bamboo flowering event. Further support for this mechanism was found in FS and GS microhabitats, where −R filters (live understorey simulation) were sufficient to significantly reduce seedling emergence (Fig. 3a,b). Several other studies have demonstrated this light quality dependence of seed germination in some forest species (Vázquez-Yanes & Smith, 1982; Vázquez-Yanes *et al.*, 1990; Bell *et al.*, 1999; Pearson *et al.*, 2003). Our results are consistent with these studies and suggest that R:FR ratios may be an important germination cue for overstorey species in this ecosystem, and the unique nature of the bamboo flowering event may create a short-lived opportunity for extensive overstorey germination.

At the same time, the lack of germination response under live bamboo with −FR filters (senescent understorey simulation) suggests that high R:FR ratios alone are not sufficient to explain the stimulatory effects on seed germination observed in the FS and GS microhabitats (Fig. 3c). There appear to be additional controls on seed germination, such that certain factors create inhibitory effects in dense live understorey microhabitats. These possibilities include reduced thermal amplitude (Vázquez-Yanes & Orozoco-Segovia, 1982; Thompson & Grime, 1983; Pearson *et al.*, 2002), reduced soil moisture, and low soil nitrate (Bell *et al.*, 1999; Daws *et al.*, 2002), all of which have been identified as additional factors controlling the seed germination response in a number of species (Ballaré, 1994). In this study, daily thermal amplitude was 2–5°C lower in FL than in FS microhabitats, with the difference being most marked in the early spring (Fig. 2). The decreased thermal amplitude could have served as an inhibitor of germination in live understorey microhabitats, thereby preventing a germination response to increased R:FR ratios under the −FR filters. The results of the laboratory incubations strongly support the proposition that light quality and thermal amplitude interact as germination cues for *N. obliqua*, with greatly enhanced germination under conditions of high R:FR ratios and fluctuating temperature (Fig. 4). Although these results contrast with those of other studies which demonstrated that large-seeded species showed little or no response to changes in light quality (Pearson *et al.*, 2002; Jankowska-Blaszcuk & Daws, 2007), it appears that *N. obliqua* is most responsive to multiple cues indicating auspicious conditions for germination. Given that *Nothofagus* spp. do not form a persistent seed bank in field conditions (Cuevas & Arroyo, 1999), and the infrequent nature of these flowering events, these results suggest that the overstorey species in this ecosystem are restricted in their germination window, given the necessity of a concurrence of both high R:FR ratios and alternating temperatures in order to elicit a strong germination response.

Seedling growth in the different microhabitats in the first season after emergence supports the idea that the most favorable conditions are found in microhabitats with a senescent understorey. The significant increase in internode elongation in *N. obliqua* in live understorey microhabitats (FL) could be seen as part of the classic 'shade avoidance' response (Morgan & Smith, 1976; Ballaré, 1994) that can be elicited by multiple signals of the light environment typical of shaded areas: low R:FR ratios perceived by phytochromes and low irradiance perceived by a variety of photoreceptors (Ballaré, 1994). In

Table 3 Mean (SE) *Nothofagus obliqua* seedling growth in the forest microhabitats at the end of the first growing season

	Growth parameter					
Microsite	Internode length (cm)	Number of leaves	Height (cm)	PAR daily dose[1]	% PAR	R:FR[2]
FL	1.09 (0.03) ^{a}	3.21 (0.15) ^{a}	3.49 (0.14)	4.46×10^4	2.22	0.18 (0.04)
FS	0.83 (0.05) ^{b}	3.81 (0.18) ^{a}	3.14 (0.23)	3.22×10^5	16.03	0.52 (0.06)
GS	0.76 (0.04) ^{b}	5.06 (0.29) ^{b}	3.74 (0.23)	6.91×10^5	34.4	0.81 (0.03)
GR	0.77 (0.02) ^{b}	5.03 (0.40) ^{b}	3.8 (0.39)	2.01×10^6	100	0.85 (0.023)
P	< 0.0001	< 0.0001	0.2181	–	–	–

FL, forest with live bamboo understorey; FS, forest with senesced bamboo understorey; GS, canopy gaps with senesced bamboo; GR, gaps where senesced bamboo culms were manually removed.

Photosynthetically active radiation (PAR) daily dose, % PAR (relative to the GR), and midday red:far-red (R:FR) values of representative microhabitats are reported. *P*-values from one-way ANOVA are shown. Different superscript letters indicate different means according to a post-hoc Tukey Honest Significant Difference (HSD) test.

[1] Expressed in µmol m^{-2}, integrated between 10:00 and 16:00 h.

[2] Mean values (SE) from measurements of two replicated microhabitats.

microhabitats in which the understorey is senescent or absent (FS, GS and GR), increased R:FR ratios were accompanied by a 3- to 10-fold increase in PAR availability (Marchesini *et al.*, in press; Table 3). In FL microhabitats, where PAR daily irradiance is *c.* 3% that of open areas (Table 3), seedlings assigned more resources to internode elongation over leaf production, while seedlings in open spaces invested more in leaf production with significantly less internode elongation (Table 3). The growth of emergent tree seedlings appears to be controlled by light as a signal to escape shade and as a limiting resource for photo-assimilate production (Ritchie, 1997; Munth & Bazzaz, 2002). In live understorey microhabitats, it appears that light quality cues promoted increased elongation, while low PAR irradiance constrained the growth response. These conflicting light effects resulted in a compensatory response such that there was no difference in seedling height among microhabitats (Table 3).

Taken together, these results support the view that *natural* variation in light quality, observed in the wake of this gregarious flowering event, can elicit plant physiological responses of sufficient magnitude to impact forest dynamics in this ecosystem. They further suggest that the principal regulation of forest regeneration in this ecosystem may be biological, acting via alterations in microenvironmental characteristics of the forest floor as a result of changes in understorey cover and biomass. Future studies linking flowering events and tree masting in these *Nothofagus* species (Richardson *et al.*, 2005) as well as other constraints on seedling survival including changes in granivore and herbivore pressure (Abe *et al.*, 2001; Caccia *et al.*, 2006; Kitzberger *et al.*, 2007; Raffaele *et al.*, 2007), are necessary to fully understand the ecological consequences of this episodic flowering event for forest regeneration. Nevertheless, the extensive death of the understorey produced changes in the spectral composition of the light environment and thermal amplitude, which, combined with the photoblastic germination response of *N. obliqua*, opened a sporadic window of opportunity for regeneration of overstorey tree species. This study demonstrates that this infrequent event is an important component controlling forest regeneration in this ecosystem.

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