Plant Ecology (2006) 185:29–39
DOI 10.1007/s11258-005-9081-4

Carbon sink limitation and frost tolerance control performance of the tree *Kageneckia angustifolia* D. Don (Rosaceae) at the treeline in central Chile

Frida I. Piper, Lohengrin A. Cavieres*, Marjorie Reyes-Díaz and Luis J. Corcuera
*Departamento de Botánica, Facultad de Ciencias Naturales y Oceanográficas, Universidad de Concepción, Casilla 160-C, Concepción, Chile; *Author for correspondence (phone: +56-41-203846; fax: +56-41-246005; e-mail: lcaviere@udec.cl)*

Received 1 December 2004; accepted in revised form 17 November 2005

Key words: Altitudinal gradient, Carbon balance, Growth limitation, Low temperature resistance

Abstract

The decrease in temperature with increasing elevation may determine the altitudinal tree distribution in different ways: affecting survival through freezing temperatures, by a negative carbon balance produced by lower photosynthetic rates, or by limiting growth activity. Here we assessed the relative importance of these direct and indirect effects of altitudinal decrease in temperature in determining the treeline in central Chile (33°S) dominated by *Kageneckia angustifolia*. We selected two altitudes (2000 and 2200 m a.s.l.) along the treeline ecotone. At each elevation, leaf non-structural carbohydrates (NSC) and gas exchange parameters were measured on five individuals during the growing season. We also determined the cold resistance of *K. angustifolia*, by measuring temperatures that cause 50% seedling mortality (LT_{50}) and ice nucleation (IN). No differences in net photosynthesis were found between altitudes. Although no differences were detected on NSC concentration on a dry matter basis between 2000 and 2200 m, when NSC concentration was expressed on a leaf area basis, higher contents were found at the higher elevation. Thus, carbon sink limitations may occur at the *K. angustifolia*'s upper altitudinal limit. For seedlings derived from seeds collected at the 2200 m, LT_{50} of cold-acclimated and non-acclimated plants were −9.5 and −7 °C, respectively. However, temperatures as low as −10 °C can frequently occur at this altitude during the end of winter. Therefore, low temperature injury of seedlings seems also be involved in the treeline formation in this species. Hence, a confluence of global (carbon sink limitation) and regional (freezing tolerance) mechanisms explains the treeline formation in the Mediterranean-type climate zone of central Chile.

Introduction

The treeline, defined as the upper limit of altitudinal tree distribution (Körner 1998, 1999), is one of most conspicuous vegetation boundaries worldwide. Treeline environments are generally characterized by harsh climatic conditions, which restrict growth, reproduction, and many metabolic functions to short favorable periods (Hinckley et al. 1984). Many factors have been proposed to explain the formation of treelines (Tranquillini 1979; Stevens and Fox 1991; Körner 1998), but the exact physiological mechanisms involved are not clearly identified (Hoch et al. 2002).

The most evident environmental changes with elevation are the decrease in temperature and growing season length (Körner 2003). The effects of the altitudinal decrease in temperature on biological processes related with the treeline set-up can be direct or indirect (Cavieres and Piper 2004). Possible direct mechanisms include severe injury or killing of tree seedlings by freezing temperatures:

the limit should be reached at the altitude where the tissues can still survive ambient temperatures. Treeline habitats in temperate latitudes are seasonally harsh, and trees acclimate to this condition by becoming dormant during winter. Hence, temperate treelines are rarely determined by injury of freezing temperatures (Tranquillini 1979; Körner 1998). However, episodic drops of temperature during spring or summer in temperate climates can be particularly dangerous, because plants are in an active growth state (Alberdi and Corcuera 1991). Freezing injury limited treelines are more commonly found in tropical treelines where trees cannot avoid the harsh environment of high elevation tropical night by becoming dormant (Cabrera 1996; Cavieres and Piper 2004). All studies that had evaluated the importance of freezing injury in treeline formation have been carried out in adult individuals (e.g., Rada et al. 1987, 2001; Squeo et al. 1991; Goldstein et al. 1994; Cavieres et al. 2000). However, it is well known that seedlings are more susceptible to freezing temperatures than adults (Sakai and Larcher 1987), and some studies suggest that some treelines in temperate zones can be determined by processes occurring at the seedling level (Ferrar et al. 1988; Germino and Smith 1999, 2002).

Among the indirect effects, two alternative hypotheses have been proposed to explain treeline elevation. Firstly, it has been suggested that the altitudinal decrease in temperature depresses photosynthetic rates, limiting carbon gain (Boysen-Jensen 1949; Stevens and Fox 1991). Hence, the treeline will occur at the elevation where annual carbon gains cannot compensate respiration losses. However, gas exchange data provide little empirical evidence that insufficient photosynthetic activity during the growing season or excess respiratory losses at the higher altitudes can explain treelines in temperate zones (Tranquillini 1979; Körner and Cochrane 1985; Körner 1998).

On the other hand, since growth processes tend to be more sensitive to low temperature than photosynthesis (Grace et al. 2002), it has been suggested that cell and tissue formation will be limited with the elevational decrease in temperature (Körner 1998). The latter implies a low temperature threshold for sink activity (growth) rather than a limitation on source activity (insufficient carbon gain). Thus, even though both hypotheses propose that the treeline is determined by a temperature effect on carbon balance, the causal direction of the processes involved is opposite.

The concentration of non-structural carbohydrates (NSC) has been widely used to assess the balance between carbon acquisition (sources) by photosynthesis and carbon demand (sinks) for growth and respiration processes (Oleksyn et al. 2000; Li et al. 2002; Hoch et al. 2002; Körner 2003; Hoch and Körner 2003). When photosynthesis is limited, it is expected that NSC concentration will decrease (acquisition < demand). Conversely, if growth or respiration is the critical process limited by temperature, the NSC pool size should increase (acquisition > demand) (Li et al. 2002; Höch et al. 2002; Höch and Körner 2003). Therefore, an assessment of the NSC pool size across an altitudinal gradient should be an informative contribution to the carbon source–sink debate with respect to treelines (Höch et al. 2002).

This paper reports the direct and indirect effects of temperature on the small tree *Kageneckia angustifolia* growing at treeline in the Mediterranean-type climatic region of central Chile. Direct effects were studied by the resistance of seedlings to freezing temperatures, while indirect effects were studied by considering the relative importance of the carbon and growth limitation hypotheses. If the source:sink ratio in *K. angustifolia* increases with altitude (growth limitation), the size of the NSC pool in *K. angustifolia* should increase as the altitudinal limit of tree growth is reached. On the other hand, if the source/sink ratio decreases with altitude (carbon gain limitation), the opposite trend is expected. To address these hypotheses, environmental and gas exchange parameters were measured and the foliar NSC concentration was determined during the growing season at two altitudes in the treeline ecotone of central Chile.

Methods

Studied species

Kageneckia angustifolia D. Don (Rosaceae) is a dioecious tree, endemic to the Mediterranean-type climate zone of central Chilean (Rodríguez et al. 1983). At 32°–33° S, this species occupies an ecotonal position between the montane forest, in which it is the single tree species present, and the high Andean vegetation, forming the treeline at ca.

2200 m a.s.l. (Rundel 1981). *K. angustifolia* can be described as a small tree species that forms open woodlands, with open canopies that do not produce sharp microclimatic differences between open areas and beneath tree canopy (Peñaloza et al. 2001). Additionally, this species undergo partial foliage loss during the summer season (León 1993; Peñaloza et al. 2001).

Study site

The field study was carried out with adults of *Kageneckia angustifolia* growing at the treeline ecotone at Farellones in central Chile, during the 2002–2003 growing season. On a west-facing slope, two sites of similar inclination (ca. 10%) were chosen at 2000 and 2200 m a.s.l. (33°21′00″ S; 70°19′21″W and 33°21′44″S; 70°19′13″W, respectively). We selected these two sites on the same slope in order to control for strong effects of slope aspect and inclination, substrate and mesoclimate. On the study area mean monthly temperature ranges from a maximum of 20 °C in January to a minimum mean of −0.8 °C in July. Freezing temperatures occur during 181 days per year with extreme temperatures as low as −10 °C occurring during winter and early spring (Santibáñez and Uribe 1992). The mean annual precipitation is 445 mm, and the summer drought period extends for 5 months (op.cit.).

Gas exchange

Five healthy trees of similar crown size were selected at each altitude for gas exchange measurements. On each tree, a one-year-old leaf on a branch of western aspect was selected. On each leaf we measured net photosynthesis, stomatal conductance, foliar temperature, and photosynthetically active radiation (PAR) using an infrared gas analyzer CIRAS-1 (PP Systems, Hitchin, England). Measurements were carried out at three periods during the 2002–2003 growing season: early growing season (December), mid growing season (January) and late growing season (March). For each period, gas exchange measurements were carried out during two consecutive days, one for each altitude. Measurements were performed at midday because preliminary data indicated that maximum photosynthetic rates occurred at this time. Midday measurements consisted of two consecutive cycles of measurements on selected trees, enabling us to account for instantaneous variations in weather conditions affecting the photosynthetic performance of trees (e.g., transient overcast conditions). A full daily cycle of measurements was added in March: as this is the driest month of the year, midday photosynthesis is unlikely to be representative of daily carbon gain, due to stomata closure. The daily cycle measurements in March were performed between 3:00 and 23:30.

Non-structural carbohydrates (NSC) determinations

On the same five trees and branches used in the gas exchange measurements, we additionally selected six one-year-old leaves for NSC analyses. We determined NSC concentration at three dates during the 2002–2003 growing season; mid growing season (January), late growing season (March) and onset of winter (May). We delayed the sampling for NSC measurements respect to gas exchange measurements because previous studies (Hoch and Körner 2003) indicated that it is at the end of the growing season when the ‘growth limitation hypothesis’ can be better observed. On each of these periods, samples were taken in a similar way to that employed in gas exchange measurements; that is on consecutive days for each altitude and consecutive cycles on the trees at each site. General weather conditions were the same for the two consecutive days at each sampling period. Like gas exchange measurements, a full daily cycle of NSC concentration was analyzed in March. Leaves were immersed in liquid N_2 immediately after removal, and kept there until the analysis. Leaves were thawed at room temperature for 10 min. prior to analysis, and their fresh weight recorded. One third of each sample (1–2 leaves) was used for dry weight measurements after drying at 70 °C for 2 days. The remaining tissue was used for carbohydrate analysis. Samples were analyzed for total soluble sugars and starch using ethanol and perchloric acid method (Hansen and Moller 1975).

Total soluble sugars (TSS) were extracted from fresh leaf tissue in 86% v/v ethanol with overnight agitation. TSS concentration was determined spectrophotometrically by the Resorcinol method (Roe 1934) at a wavelength of 520 nm, using sucrose as standard. Starch was extracted with 52% (v/v) perchloric acid and agitation for 48 h. The protocol for starch determination in the extract was similar to that used for total soluble sugars, but using glucose as standard. The Hansen and Moller (1975) method usually overestimates starch levels because carbohydrates other than starch can be extracted during the process (Marshall 1985; Rose et al. 1991). Hence, new estimations of carbohydrates were performed using less concentrated perchloric acid. For that, we repeated the extraction for the leaf samples taken in March in the afternoon, but now using 35% (v/v) of perchloric acid. By comparing both data a correction factor was obtained and then applied to the entire data set. Therefore, perchlorate extractable carbohydrates were corrected to yield starch concentrations and are expressed in their corrected form throughout this article. The NSC concentration was calculated as the sum of starch and TSS concentrations.

Because leaf tissues could increase in density with increasing altitude (e.g., have a lower specific leaf area), NSC concentration in leaves on a dry matter basis could be 'diluted' by structural compounds (Hoch et al. 2002; Körner 2003). To solve this problem, specific leaf area (SLA) was estimated in six leaves per tree at each elevation. Leaf areas were estimated by scanning leaves and estimating their area with the software SigmaScan®, whereas dry-weight of leaves was obtained after drying them at 70 °C for 48 h. Hence, all sugars concentrations are expressed per unit of leaf area, as well as per unit of dry weight (D.W.).

Resistance to freezing temperatures

In March of 2004, fruits were collected at each altitude and brought to the laboratory, where their seeds were sown in small pots containing a 1:1 mixture of sand and peat. Seeds were germinated in a growth chamber (Bioref, Santiago, Chile) under a 15/9 h (light/dark) photoperiod provided by 80 W incandescent lamps and a 25/10 °C (day/night) temperature regime. When seedlings had two cotyledons and two expanded leaves, they were used for thermal analysis (ice nucleating and freezing temperatures determinations) and LT_{50} measurements (temperatures that cause 50% seedling mortality). In order to assess the potential of freezing resistance for seedlings during winter conditions, 21 days before the beginning of analyses, one half of the seedlings were acclimated at 4 °C (cold acclimated plants) while the rest were kept at 25/10 °C (non-acclimated plants). It has been shown that several plant species display freezing resistance responses after a cold-acclimation period of 20 days at 4 °C (Levitt 1980; Alberdi and Corcuera 1991; Zúñiga-Feest et al. 2003; Pérez-Torres et al. 2004). For thermal analysis, at each out of five replicates, one expanded leaf was removed and attached to a thermocouple (Gauge 30 Copper-constantan thermocouples; Cole Palmer Instruments Co., Vernon Hills, Illinois, USA), and immediately enclosed in a small, tightly closed cryotube to avoid changes in tissue water content. Temperature was continuously monitored (1 measurement per second) with Daq view program and connected to a multi-channel temperature terminal panel (Strawberry Tree Inc. Sunnyvale, California, USA). The tubes were placed in a cryostat and the temperature was lowered from 0 to −15 °C at a rate of 2 °C/h. The temperature at the initiation of the exotherm corresponds to the ice nucleation (IN), while the highest point of the exotherm represents the freezing temperature (FT) of the water in the apoplast (including symplastic water driven outwards by the water potential difference caused by apoplastic ice formation) (Larcher 2003). Freezing resistance was evaluated as percentage survival of seedlings after freezing. Eight whole seedlings were frozen for 2 h at temperatures between 0 and −15 °C in a stainless steel container submerged in a cryostat. At each temperature, plants were removed and thawed overnight at 4 °C. Survival of seedlings during the next 10 days was monitored at 25/10 °C (day/night). LT_{50} was the average temperature of the eight replicates at which 50% of the plants died.

Statistical analyses

Repeated measurement two-way ANOVAs were used to test differences in photosynthesis and NSC content between dates and sites. In both cases, the two consecutive cycles performed at each elevation, and at each sampling date, were averaged. The same statistical test was used to evaluate differences in the daily cycles taken in March for NSC content and photosynthesis. IN, FT, and LT_{50} of seedlings were compared between altitudes and acclimation treatments with a two-way ANOVA.

Results

Gas exchange

Net photosynthesis did not differ between altitudes ($F_{1,36} = 1.09$; $p = 0.311$), despite higher PAR and foliar temperature at the high elevation site (Figure 1). However, net photosynthesis differed between sampling dates ($F_{2,36} = 29.12$; $p < 0.001$) showing the lowest level in January. The lower photosynthesis observed in January with respect to the other dates, was probably related to lower stomatal conductance at this date (Figure 1).

The daily cycles of net photosynthesis taken in March differed between hours, but not between altitudes (Table 1a). During the morning photosynthesis was higher at 2200 m, but in the afternoon it was higher at 2000 m (Figure 2). Nonetheless, these differences cannot be explained either by PAR levels or by foliar temperatures (Figure 2). Differences in stomatal conductance cannot also be related with the observed difference in photosynthesis (Figure 2). Stomatal conductance showed a bimodal tendency at 2000 m, probably due to a stomatal closure at midday with greater values during the morning and the afternoon, reaching the maximum by 16:00 (Figure 2). At the higher site, stomatal conductance was low at night and early morning hours, reaching its maximum at midday.

NSC concentrations

Seasonally, total soluble sugars (TSS) and starch showed similar patterns of variation as they were expressed on a dry matter basis, without altitudinal differences among them (data not shown). Repeated measures ANOVA indicated that NSC concentration did not differ either between altitudes ($F_{1,28} = 1.66$; $p = 0.218$) or between sampling dates ($F_{2,28} = 1.87$; $p = 0.173$). However, when data were expressed on a leaf area basis, TSS and starch were consistently higher at the higher elevation (Figure 3). The highest difference in both compounds occurred in May, when TSS and starch levels were 22% higher at 2200 m. Theses differences were reflected in the NSC levels, which were significantly higher at 2200 m in all sampling dates ($F_{1,28} = 93.28$; $p < 0.0001$).

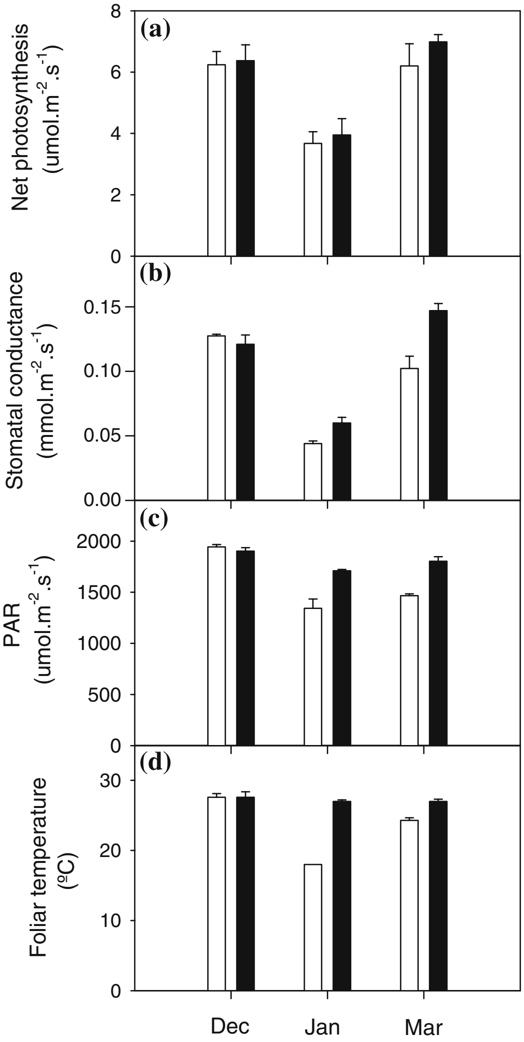

Figure 1. Seasonal variation in photosynthesis for individuals of the tree *Kageneckia angustifolia* at different dates during the 2002–2003 growing season at two altitudes. Net photosynthesis (a), Stomatal conductance (b), Photosynthetically active radiation (PAR) (c), and Foliar temperature (d). White bars correspond to 2000 m and black bars to 2200 m. Error bars indicate standard error.

Table 1. Repeated-measurement ANOVA for the daily cycle of net photosynthesis (a), NSC concentration on a dry matter basis (b) and NSC concentration on a leaf area basis (c).

Factor	df	*F*	*p*
(a)			
Altitude	1	0.08	0.79
Time	8	157.04	< 0.001
Altitude*time	8	33.09	< 0.001
Error	64		
(b)			
Altitude	1	8.17	0.021
Time	4	8.93	< 0.001
Altitude*time	4	1.52	0.22
Error	32		
(c)			
Altitude	1	504.6	< 0.001
Time	4	10.0	< 0.001
Altitude*time	4	2.4	0.08
Error	32		

The daily cycles of NSC showed that in both altitudes, NSC levels decreased during the night and increased during the day. Although statistical analysis of the daily cycles of NSC concentration on a dry matter basis indicated differences between elevations and time of day (Table 1b), *a posteriori* Tukey-HSD tests showed that altitudinal differences were due to the values obtained at 7:00, which was higher at the lower elevation. Thus, no strong altitudinal effect can be inferred from the daily cycle of NSC on a dry matter basis on March. However, when NSC was expressed per leaf area unit, NSC concentrations were significantly higher at 2200 m (Figure 4, Table 1c). Starch and TSS levels were higher for 2200 m for the complete cycle as well, with the exception of TSS level at 17:00, who was similar for both altitudes.

Freezing resistance mechanism

Two-way ANOVAs indicated that IN temperature and FT of seedlings from 2200 m were significantly lower than for seedling from 2000 m ($F_{1,19} = 63.102$; $p < 0.001$; $F_{1,19} = 51.98$; $p < 0.001$, for IN and FT, respectively). Freezing temperatures (FT) were significantly lower in cold-acclimated plants for seedlings of both altitudes (Figure 5) ($F_{1,19} = 60.84$; $p < 0.001$). In contrast, for IN, only seedlings from 2200 m showed a temperature significantly lower ($F_{1,19} = 12.2$; $p = 0.003$) for non-acclimated than for cold-acclimated seedlings. LT_{50} was lower for seedlings from 2200 m ($F_{1,11} = 77.84$; $p < 0.001$), but for both

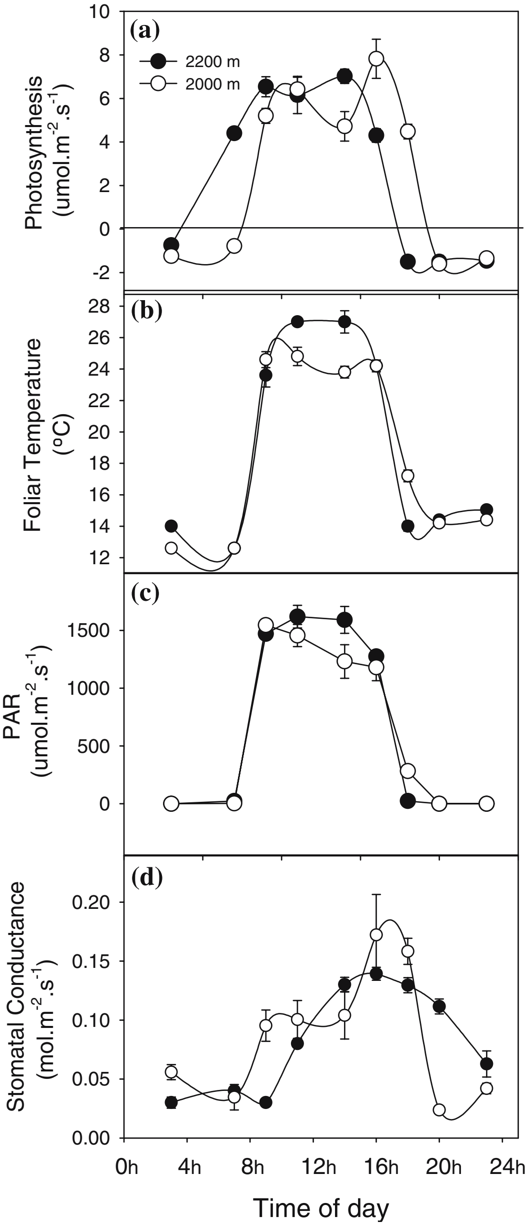

Figure 2. Daily cycle of photosynthesis of *Kageneckia angustifolia* during a typical day in March, at two altitudes. Net photosynthesis (a), Foliar temperature (b), Photosynthetically active radiation (PAR) (c) and Stomatal conductance (d). Empty circles correspond to 2000 m and black circles to 2200 m. Error bars indicate standard error.

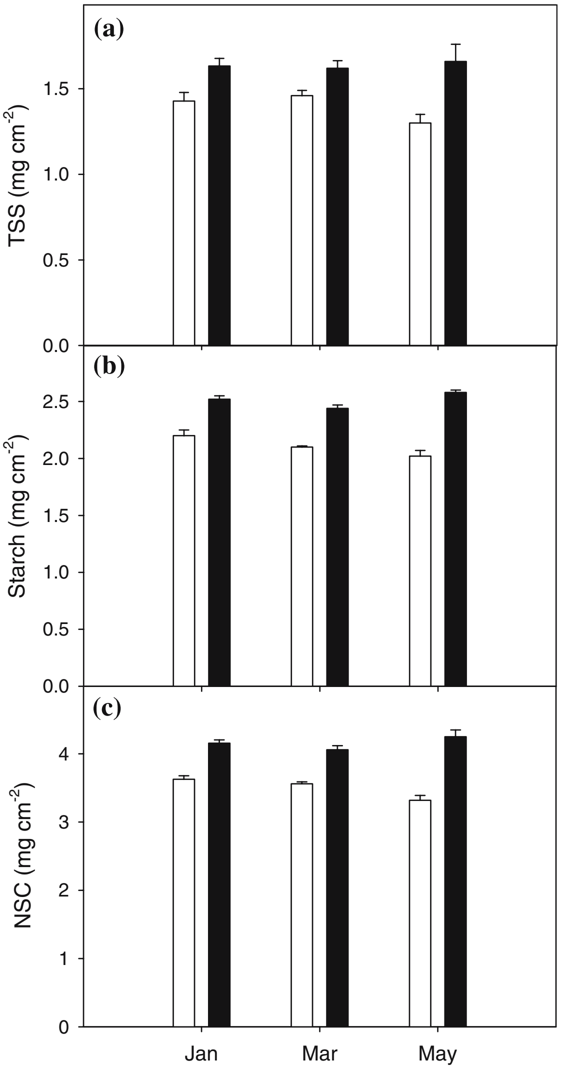

Figure 3. Seasonal variation of sugars (g/cm^2) in *Kageneckia angustifolia* during the 2002–2003 growing season at two altitudes. Total soluble sugars (TSS) (a), starch (b) and non-structural carbohydrates (NSC) (c). White bars correspond to 2000 m and black bars to 2200 m. Error bars indicate standard error.

sites seedlings died at temperatures higher than IN and FT (Figure 5). Seedlings from both altitudes responded to cold-acclimation by lowering their LT_{50} ($F_{1,11} = 228.55$; $p < 0.001$).

Discussion

Our results suggest that *Kageneckia angustifolia*'s treeline in central Chile can be explained by a combination of direct and indirect effects of low temperature. Among the indirect effects, our results for photosynthesis and NSC do not unequivocally support the carbon source limitation hypothesis, because no differences in net photosynthesis of *K. angustifolia* were found between altitudes across the treeline. This result agrees with the evidence reviewed by Körner (1998) for temperate zones, where treelines cannot be explained by the carbon source limitations. Nevertheless, some evidence for carbon gain limitations has been observed at treelines on tropical mountains (Cavieres et al. 2000).

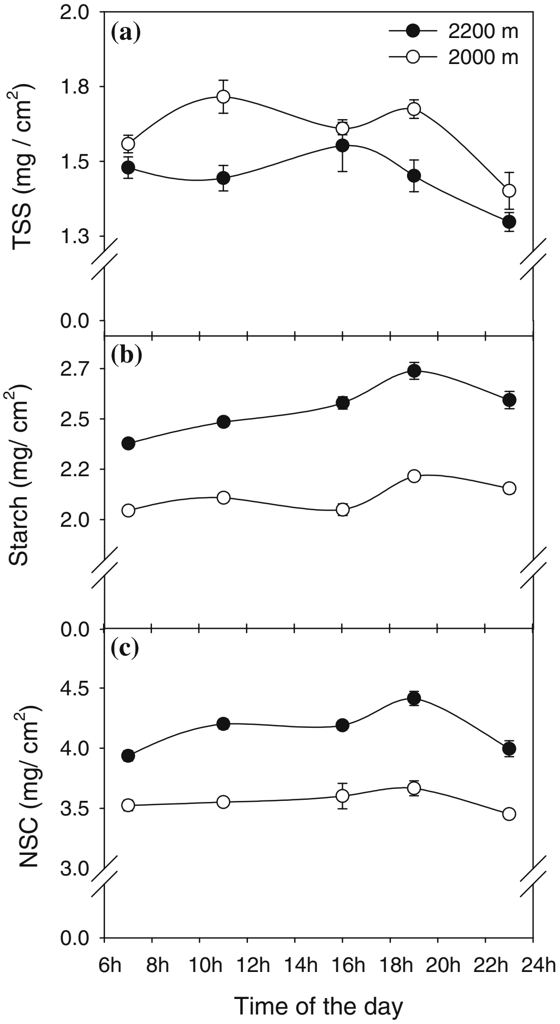

Figure 4. Daily cycle of sugars (g/cm^2) in *Kageneckia angustifolia* during a typical March day at two altitudes. Total soluble sugars (TSS) (a), starch (b) and non-structural carbohydrates (NSC) (c). The empty circles correspond to 2000 m and black circles to 2200 m. Error bars indicate standard error.

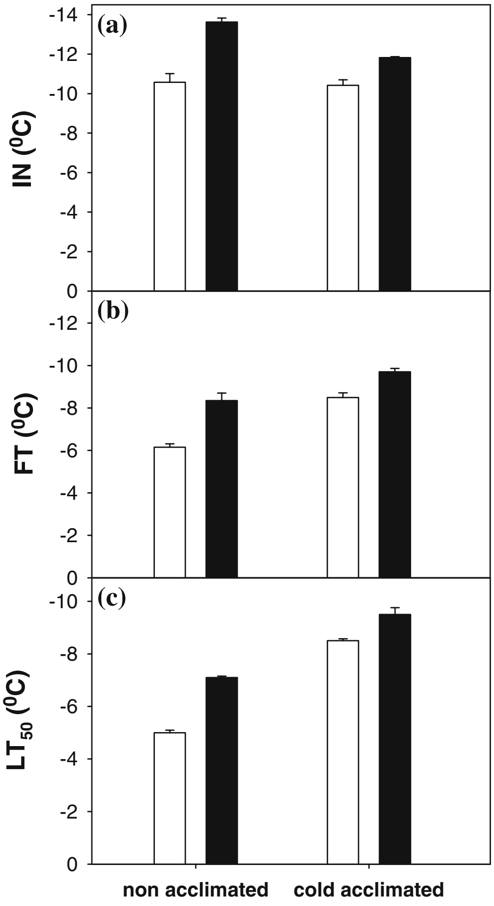

Figure 5. Freezing resistance for non-acclimated and cold-acclimated seedlings of *Kageneckia angustifolia*. Ice nucleation temperature (a), Freezing temperature (b) and Lethal temperature for 50% of seedlings (c). White bars correspond to 2000 m and black bars to 2200 m. Error indicate standard error.

Conversely, we find support for the carbon sink limitation hypothesis. While NSC concentration on a dry matter basis did not increase with altitude in any of the sampling dates, NSC concentration on a leaf area basis did, suggesting that carbon use limitations are involved in the formation of *Kageneckia angustifolia*'s treeline in central Chile.

Since its initial formulation by Körner (1998), few studies have evaluated the importance of carbon sink limitations in the formation of the upper altitudinal limit of trees (but see Hoch et al. 2002; Hoch and Körner 2003). This hypothesis is based on the fact that, unlike shrubs and forbs that can benefit from soil heat flux, trees crown are highly coupled with atmospheric temperature, which directly affect the meristematic activity. For instance, Grace et al. (2002) showed that in *Pinus sylvestris*, growth ceased at ca. 5 °C, whereas net photosynthesis runs at ca. 30% of its maximum. Likewise, Khutornoi et al. (2001) reported significantly reduced apical meristematic activity in *Pinus sibirica* at treeline elevations compared to populations from lower elevations. Additionally, tree stature and crown can produce a self-shading effect on roots, increasing the growth limitations by reducing temperature at the root level (Körner 1998; Grace et al. 2002; Körner and Paulsen 2004). Recently, Hoch et al. (2002) and Hoch and Körner (2003) studying 3 species of *Pinus* at different latitudes in the north hemisphere have yielded clear evidences of increasing non-structural carbon charging of trees as one approaches their upper altitudinal limit, supporting the carbon sink limitation hypothesis. Hence, our study constitutes a new example of treelines where carbon sink limitations are involved, and is the first carried out with an angiosperm species. Notwithstanding, in our study, the evidence of increasing non-structural carbohydrates with altitude only emerge when data were expressed on leaf area basis. This was due to an altitudinal increase in tissue density (data not shown), which produced a dilution effect when data were expressed on a dry matter basis. A similar pattern was reported by Hoch et al. (2002), where NSC in stems changed with altitude only when altitudinal increases in tissue density were accounted for.

Although sugar accumulation is also related with cryoprotection (Alberdi and Corcuera 1991), samples of *Kageneckia angustifolia* taken at its upper altitudinal limit, showed no differences between NSC content during the growing season (January and March) and the onset of winter (May). This suggests that the observed patterns are not strongly influenced by acclimation responses to winter period.

While NSC concentrations in leaves do generally respond to different weather conditions, C-storage pools in woody tissues are more stable diurnally and seasonally, hence, they could be preferred for the assessment of the whole tree carbon supply status. However, in several studies it can be observed that NSC contents of leaves are highly correlated with total carbon pool (e.g., Glerum and Balatinecz 1980; Hoch et al. 2002; Hoch and Körner 2003; Körner 2003), suggesting that they can be used as a reliable surrogate (Glerum and Balatinecz 1980). In addition, although we are aware of the lack of spatial replication at each elevation, it should be recalled that our study sites were located on the same slope, hence, strong influences of factors other than altitude (e.g., substrate differences) are unlikely.

Freezing resistance results indicated that *Kageneckia angustifolia* is a species with cold acclimation capacity that cannot survive at temperatures lower than its IN point. Non-acclimated seedlings from 2200 m showed a LT_{50} of −7 °C, while cold acclimated seedling increased their freezing resistance to −9.5 °C, which is in line with the ability of several species to increase their frost resistance after a hardening period (Bannister 1984; Sakai and Larcher 1987; Bannister et al. 1995). However, ambient temperatures lower than this threshold can severely compromise the survival of *K. angustifolia* seedlings beyond the treeline elevation.

According to Peñaloza et al. (2001), *Kageneckia angustifolia* seedling emergence occurs at the end of winter between August and September. During this period it is likely that seedlings have lost their cold acclimation capacity because daily average temperatures begin to increase and they are in an active growth state (c.f., Alberdi and Corcuera 1991). Minimum temperature records from near the 2200 m elevation, indicate that temperatures as low as −10 °C can frequently occur at ground level during late winter (Santibañez and Uribe 1992). These temperatures are lower than the LT_{50} found for non-acclimated seedling, suggesting that freezing resistance could be involved in the determination of *K. angustifolia* treeline. Indeed, even if seedlings can acclimate to cold, minimal temperatures recorded in the field are lower than those that *K. angustifolia* seedlings can survive (−9.5 °C). Estimations of LT_{100} indicated that non-acclimated seedlings of 2200 m died at −9 °C, suggesting that a single event of extremely low temperature would produce the death of all seedlings. Therefore, our results do not support the idea that in seasonal climates the resistance to low temperatures in treeline species exceeds the environmental demand (Körner 1998). Although this hypothesis needs to be further tested in the field, it has been observed that adult trees produce great amounts of viable seeds (data not shown), however, no evidence of regeneration has been found at 2200 m. Furthermore, Peñaloza et al. (2001) reported that in this species, the higher mortality of seedlings occurs in September, where soil moisture is not limiting, but events of extremely low temperature are frequent.

Seedlings are generally considered to be less frost resistance than adults (Sakai and Larcher 1987), however several species in the southern hemisphere have shown the opposite trend (Alberdi and Rios 1983; Rios et al. 1988; Bannister et al. 1995). Although there is no specific studies for *K. angustifolia*, authors have never observed evidence of frost damage (blackening in the tip of leaves) on adults at the treeline elevation. Additionally, it has been suggested that seedling location can be important in determining their susceptibility to frost damage, with seedlings growing beneath the canopy developing less resistance than seedlings growing in exposed situations (Bannister et al. 1995). For *K. angustifolia*, it has been shown that there is no microclimatic differences between open areas and beneath canopy (Peñaloza et al. 2001). Furthermore, the majority of seeds germinate beneath the canopy of adult plants, which is free of snow earlier than open areas (Peñaloza et al. 2001), suggesting that seedlings are more exposed to extreme low temperatures beneath the canopy than in open spaces between adults. Examples of frost resistance playing an important role in treeline determination have been reported only for tropical tree species (Goldstein et al. 1994; Cavieres et al. 2000). Thus, *K. angustifolia* represents an exception in terms of the importance of low temperature resistance in the treeline determination at temperate latitudes.

The low capacity of *Kageneckia angustifolia* to resist lower temperatures could be related to its biogeographical origin. The genus *Kageneckia* is endemic to South America with 3 species (Rodríguez et al. 1983), where two of them (*K. angustifolia* and *K. oblonga*) grow in the Mediterranean-type climate zone central Chile, which is characterized by mild winters compared to other temperate climates (Arroyo et al. 1995). The other species, *K. lanceolata* grows in the tropical high elevation montane forests of Peru and Bolivia, where freezing temperatures are infrequent. This suggests that there is a strong taxonomic influence (Körner and Paulsen 2004) on the treeline altitude in the Mediterranean-type climate zone of central Chile.

In conclusion, our results suggest that both, sink limitations and seedling freezing resistance are likely involved in forming the treeline of *Kageneckia angustifolia* in central Chile. While the carbon sink limitation hypothesis as been proposed as global mechanism for treeline formation (Körner 1998), it does not dismiss other, more regional influences as seems to be the case for seedling frost resistance in *K. angustifolia*.

Acknowledgements

The authors thank to Marco Molina-Montenegro and Angela Sierra for their assistance in the field, and to Alejandra Zúñiga-Feest, Valeria Neira and Alexis Estay for their assistance in the laboratory. León Bravo, Chris Lusk and two anonymous reviewers are acknowledged for their valuable criticisms and comments. Frida I. Piper has a scholarship from the Mecesup UCO 9906 Grant. This paper forms part of the research activities of the Millennium Center for Advanced Studies in Ecology and Research on Biodiversity supported by Grant No. P02-051-F ICM.

References

Alberdi M. and Rios D. 1983. Frost resistance of *Embothrium coccineum* Forst. and *Gevuina avellana* Mol. during development and aging. Acta Oecol. Oecol. Plant. 4: 3–9.

Alberdi M. and Corcuera L.J. 1991. Cold acclimation in plants. Phytochemistry 30: 3177–3184.

Arroyo M.T.K., Cavieres L., Marticorena C. and Muñoz-Schick M. 1995. Comparative biogeography of the mediterranean floras of central Chile and California. In: Arroyo M.T.K., Zedler P. and Fox M.D. (eds), Ecology and Biogeography of Mediterranean Ecosystems in Chile, California and Australia, Springer-Verlag, New York, pp. 43–88.

Bannister P. 1984. The seasonal course of frost resistance in some New Zealand pteridophytes. New Zealand J. Bot. 22: 557–563.

Bannister P., Colhoun C.M. and Jameson P.E. 1995. The winter hardening and foliar frost resistance of some New Zealand species of *Pittosporum*. New Zealand J. Bot. 33: 409–414.

Boysen-Jensen P. 1949. Causal plant geography. Biologiske Meddelelser 21: 1–19.

Cabrera H.M. 1996. Temperaturas bajas y límites altitudinales en ecosistemas de plantas superiores: respuestas de las especies al frío en montañas tropicales y subtropicales. Revista Chilena de Historia Natural 69: 309–320.

Cavieres L.A., Rada F., García-Nuñez C., Azocar A. and Cabrera H.M. 2000. Gas exchange and low temperature resistance in two tropical treeline species from the Venezuelan Andes (8° N). Acta Oecol. 21: 203–211.

Cavieres L.A. and Piper F.I. 2004. Determinantes ecofisiológicos del límite altitudinal de los árboles. In: Cabrera H.M. (ed.), Fisiología Ecológica en Plantas. Ediciones Universitarias de Valparaíso, Pontificia Universidad Católica de Valparaíso, pp. 249–265.

Ferrar P.J., Cochrane P.M. and Slatyer R.O. 1988. Factors influencing germination and establishment of *Eucalyptus pauciflora* near the alpine tree line. Tree Physiol. 4: 27–43.

Germino M.J. and Smith W.K. 1999. Sky exposure, crown architecture, and low-temperature photoinhibition in conifer seedlings at alpine treeline. Plant Cell Environ. 22: 407–415.

Germino M.J. and Smith W.K. 2002. Conifer seedling distribution and survival in an alpine-treeline ecotone. Plant Ecol. 162: 157–168.

Glerum C. and Balatinecz J.J. 1980. Formation and distribution of food reserves during autumn and their subsequent utilization in jack pine. Can. J. Bot. 58: 40–54.

Goldstein G., Meinzer F.C. and Rada F. 1994. Environmental biology of a tropical treeline species, *Polylepis sericea*. In: Rundel P.W., Smith A.P. and Meinzer F.C. (eds), Tropical Alpine Environments, Plant Form and Function, Cambridge University Press, Cambridge, pp. 129–149.

Grace J., Berninger F. and Nagy L. 2002. Impacts of climate change on the tree line. Ann. Bot. 90: 537–544.

Hansen J. and Moller I. 1975. Percolation of starch and soluble carbohydrates from plant tissue for quantitative determination with anthrone. Analytical Biochem. 68: 87–94.

Hinckley T., Goldstein G., Meinzer F. and Teskey R. 1984. Environmental constraints at arctic, temperate-maritime and tropical treelines. In: Turner H. and Tranquillini W. (eds), Establishment and Tending of Sub-alpine Forest: Research and Management, Proc. 3rd IUFRO Workshop. Eidg. Anst. Forstl. Vesuchswes, Berlin, pp. 21–30.

Hoch G. and Körner C. 2003. The carbon charging of pines at the climatic treeline: a global comparison. Oecologia 135: 10–21.

Hoch G., Popp M. and Körner C. 2002. Altitudinal increase of mobile carbon pools in *Pinus cembra* suggest sink limitation of growth at the Swiss treeline. Oikos 98: 361–374.

Khutornoi O.V., Velisevich S.N. and Vorob'ev V.N. 2001. Ecological variation in the morphological structure of the crown in the Siberian stone pine at the timberline. Russian J. Ecol. 32: 393–399.

Körner C. 1998. A re-assessment of high elevation treeline positions and their explanation. Oecologia 115: 445–459.

Körner C. 1999. Alpine Plant Life: Functional Plant Ecology of High Mountain Ecosystems. Springer, New York.

Körner C. 2003. Carbon limitation in trees. J. Ecol. 91: 4–17.

Körner C. and Cochrane P.M. 1985. Stomatal responses and water relations of *Eucalyptus pauciflora* in summer along an elevational gradient. Oecologia 66: 443–455.

Körner C. and Paulsen J. 2004. A world-wide study of high altitude treeline temperatures. J. Biogeogr. 31: 713–732.

Larcher W. 2003. Physiological Plant Ecology: Ecophysiology and Stress Physiology of Functional Groups. Springer-Verlag, Telos.

León P. 1993. Efecto del espaciamiento entre plantas en la producción de frutos y semillas en *Kageneckia angustifolia* D. Don., un árbol dioico del Bosque Esclerófilo montano de

Chile central. Master Thesis; Facultad de Ciencias Universidad de Chile, Santiago, Chile.

Levitt J. 1980. Responses of Plants to Environmental Stresses, Vol I. Chilling, Freezing and High Temperature Stresses. Academic Press, New York.

Li M.H., Hoch G. and Körner C. 2002. Source/sink removal affects mobile carbohydrates in *Pinus cembra* at the Swiss treeline. Trees 16: 331–337.

Marshall J.D. 1985. Carbohydrate status as a measure of seedling quality. In: Duryea M.L. (ed.), Proceedings: Evaluating Seedling Quality: Principles, Proceedings and Predictive Abilities of Major Tests. Workshop held October 16–18, 1984. Forest Research Laboratory, Oregon State University, Corvallis.

Oleksyn J., Zytkowiak R., Karolewski P., Reich P.B. and Tjoelker M.G. 2000. Genetic and environmental control of seasonal carbohydrate dynamics in trees of diverse *Pinus sylvestris* populations. Tree Physiol. 20: 837–847.

Peñaloza A., Cavieres L.A., Arroyo M.T.K. and Torres C. 2001. Intra-specific nurse effect of *Kageneckia angustifolia* D. Don (Rosaceae) and its effect on seed germination and seedling survival in the montane sclerophyllous forest of central Chile. Revista Chilena de Historia Natural 74: 539–548.

Pérez-Torrez E., Dinamarca J., Bravo L.A. and Corcuera L.J. 2004. Responses of *Colobanthus quitensis* (Kunth) Bartl. to high light and low temperature. Polar Biol. 27: 183–189.

Rada F., Goldstein G., Azócar A. and Torres F. 1987. Supercooling along an altitudinal gradient in *Espeletia schultzii*, a caulescent giant rosette species. J. Exp. Bot. 38: 491–497.

Rada F., García-Núñez C., Boero C., Gallardo M., Hilal M., González J., Prado F., Liberman-Cruz M. and Azócar A. 2001. Low-temperature resistance in *Polylepis tarapacana*, a tree growing at the highest altitudes in the world. Plant Cell Environ. 24: 377–381.

Rios D., Mezza-Basso L., Guarda P., Percizzo G. and Alberdi M. 1988. Frost hardiness and carbohydrate changes in leaves of *Nothofagus dombeyi* (Mirb.) Oerst. At various ontogenetic stages. Acta Oecol. Oecol. Plant. 9: 135–144.

Rodríguez R., Matthei O. and Quezada M. 1983. Flora Arbórea de Chile. Editorial de la Universidad de Concepción, Concepción, Chile.

Roe J.H. 1934. A photometric method for the determination of fructose and urine. J. Biol. Chem. 107: 15–32.

Rose R. et al. 1991. Starch determination by perchloric acid vs. enzymes: evaluating the accuracy and precision of six colorimetric methods. J. Agric. Food Chem. 39: 2–11.

Rundel P. 1981. The matorral zone on central Chile. In: Di Castri F., Goodall D.W. and Specht R.L. (eds), Mediterrancan-Type Shrublands., Elsevier Scientific Pub., Amsterdam, The Netherlands, pp. 175–201.

Sakai A. and Larcher W. 1987. Frost Survival of Plants. Responses and Adaptation to Freezing Stress. Springer, Berlin.

Santibañez F. and Uribe J.M. 1992. Agroclimatología de Chile Central: Regiones V y Matropolitana. Universidad de Chile, Facultad de Ciencias Agrarias y Forestales, Santiago.

Squeo F.A., Rada F., Azócar A. and Goldstein G. 1991. Freezing tolerance and avoidance in high tropical Andean plants: is it equally represented in species with different plant height? Oecologia 86: 378–382.

Stevens G.C. and Fox J.F. 1991. The causes of treeline. Annu. Rev. Ecol. Systematics 22: 177–191.

Tranquillini W. 1979. Physiological Ecology of the Alpine Timberline. Tree existence at high altitudes with special reference to the Europan Alps. Springer, Berlin.

Zúñiga-Feest A., Inostroza P., Vega M., Bravo L.A. and Corcuera L.J. 2003. Sugars and enzyme activity in the grass *Deschampsia antarctica*. Antarctic Sci. 15: 483–491.