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Tree age and bark thickness as traits linked to frost ring probability on *Araucaria araucana* trees in northern Patagonia

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ABSTRACT

Frost events may damage the cambium and consequently the newly produced tracheids whose cell walls have not yet completed their lignifications, leading to the formation of frost rings. This study deals with the presence of frost rings in *Araucaria araucana* trees according to cambial age and bark thickness, under the assumption that these factors may be involved in physical or physiological mechanisms that increase resistance to freezing temperatures that impact the cambial tissue. The study was conducted in northern Patagonia at two sites of contrasting geomorphology, and therefore potentially associated with a differential degree of exposure to extreme cold. Wood plus bark cores were extracted from main stems at two heights from the ground and from each of the four cardinal point directions for 30 individuals per site. A Linear Mixed Model and a Generalized Linear Mixed Model were applied in order to relate the bark thickness and the frequency of frost rings in accordance with the different sampling points on the stem. It was observed that as bark becomes thicker with cambial age, the frequency of frost rings decreases, indicating a possible thermal-induced mechanism of bark protection. Consequently, there is an increase in the presence of frost rings at the younger stages of tree life. Although the mechanisms of cold hardiness in trees can be complex, including aspects of the tree physiology, our data indicated that as tree age increases, the thickness of the bark is higher, resulting in a potential effect of isolation and passive protection against the harmful effects of frosts. This mechanism may be relevant in the ecology, conservation and management of forests faced with extreme variability in future climate and changing scenarios.

1. Introduction

Global warming in the twentieth century is considered one of the factors responsible for recent changes in the frequency and intensity of climate extremes (IPCC, 2007). Since predictions of climate change include an increase of temperature in temperate latitudes, a consequence of this phenomenon can be linked to a loss of acclimation to low growth temperatures, meaning a greater risk of frost damage during periods of active plant development (Cannell and Smith, 1986; Inouye, 2000; Augspurger, 2009). Extremely low temperatures are an important limiting factor for plant production and their distribution in large areas of the world, since two thirds of the world's landmass is annually subjected to temperatures below the freezing point (Lärcher, 2001). As low temperature stress impairs metabolic processes and dry matter production with different degrees of reversibility, frost action over plants has significant implications on the equilibrium of native vegetation, as well as high-yield crops (Lärcher, 1981). Based on these facts, it becomes relevant a better understanding of how plants may react to such potential changes induced by frosts.

Despite the fact that plants possess mechanisms to resist the effects of low temperatures on their development (e.g., cortical isolation, biochemical and enzymatic reactions, mitochondria rate respiration, increase in solute cell contents, etc.—see Lyons and Raison, 1970; Sakai and Lärcher, 1987; Hasanuzzaman et al., 2013), a freezing event may cause injuries in the cambial tissue, giving rise to two possible phenomena: (1) death of the meristems with consequent death of the plant, and (2) partial death of the cambial tissue and its regeneration after frost. Wood injuries derived

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by frosts were initially recognized by Rhoads (1923) as growth rings with anatomical pathologies caused by freezing temperatures, and later defined as frost rings signaled by cell morphology, shape and size that vary from the normal pattern (Kaennel and Schweingruber, 1995). Anatomically, the formation of ice crystals in the cambium zone results in freezing-altered cell wall thickness, variations in the matrix of the cellulose/hemicellulose/lignin contents and, moreover, in deformed or collapsed cells with deposits of dense material in their inner walls (Lee et al., 2007). Consequently, frost wounds in the xylem may have collateral effects in disruptions of hydraulic efficiency and risk to trigger cavitation in vessels (Sperry and Sullivan, 1992; Martínez-Vilalta and Pockman, 2002; Willson and Jackson, 2006).

Although cold or freezing stress resistance by plants comprises many different genetic, molecular and physiological features (Sakai and Lärcher, 1987; Hughes and Alison Dunn, 1996; Beck et al., 2007), there is a growing consensus that other simple characteristics may be associated to the decrease in the tree's sensitivity to frost, such as age and bark thickness (Gurskaya and Shiyatov, 2006). Total bark thickness (functional bark + rhytidome) is particularly considered to play a primary role in the thermal protection of the vascular cambium (Stöckli and Schweingruber, 1996; Treter and Block, 2004; Payette et al., 2010). In this sense, if bark thickness increases with age, the vascular cambium becomes increasingly protected against extreme colds. Several evidences indicate that most of the frost injuries are found in the rings that are close to the pith, suggesting a higher vulnerability of cambium to frost when the tree is young, and consequently its bark is still thin (Glerum, 1975; LaMarche and Hirschboeck, 1984; Stöckli and Schweingruber, 1996; Treter and Block, 2004; Gurskaya and Shiyatov, 2006; Gurskaya, 2007; Payette et al., 2010; Kidd and Copenheaver, 2014). Consequently, in tree species with age-related bark thickness, the frequency of frost rings may vary with the cambial age, that is, from juvenile to mature wood (Payette et al., 2010).

On the other hand, Schweingruber (2007) claimed that both frequency and intensity of the frost injury varies around the stem's circumference. This could be attributed to different exposures of stems to cold air or local variations of bark thickness. Moreover, the localization of the frost injury within the growth ring makes it possible to classify the frost event in relation to the growing activity (Gurskaya, 2014). In this sense, Schweingruber (2007) pointed out that frost damage at the beginning of the growth ring could indicate an extremely cold condition prior to the growing season (winter months), while frost injury on earlywood and latewood is produced during the growing season by late and early frosts, respectively (Glerum and Farrar, 1966). Although it is very unusual, two frost events in the same growth ring have also been reported (Gurskaya and Shiyatov, 2002; Hadad et al., 2012).

Among multiple applications, frost rings offer the possibility to use them as markers to successfully cross-date tree-ring series (Glerum, 1975), to estimate the degree of plant resistance to low temperatures (Gurskaya and Shiyatov, 2002), and to construct long-term extreme cold event chronologies at large geographic scales (Gurskaya and Shiyatov, 2002; Treter and Block, 2004; Gurskaya, 2007; Hadad et al., 2012). Moreover, frost rings could indicate when a large-scale atmospheric circulation and its related weather events play a role in this particular tree-growth response at local and/or regional scales (e.g., Mock et al., 2007) and when other physical phenomena, like volcanic eruptions, may be responsible for the occurrence of widespread frost events (LaMarche and Hirschboeck, 1984; Brunstein, 1996; Hantemirov et al., 2004; Salzer and Hughes, 2007, 2010).

In the Andes of Argentina between 37°20′–40°20′ SL, and from 900 to 1800 m altitude, the temperate forests of *Araucaria araucana* (Molina) K. Koch ("pehuén") spread in an ample ecological setting from the humid Andes foothills to the dry ecotone of the Patagonian steppe (Roig, 1998; Roig and Villalba, 2008). Growing under a cold temperate climate, *A. araucana* may attain centennial-to-millennial ages and a significant bark thickness development at tree maturity (Castro, 2009). It has been argued that the thickness of this bark can efficiently protect the inner living tissues (lateral cambium) from fires and other physical injury-related factors (Veblen et al., 1996; Roig and Villalba, 2008).

Recent studies reported the presence of frost-induced damages on the growth rings of *A. araucana* trees, pointing out the potential of this species to record past extreme cold events through dendrochronological techniques (Hadad et al., 2012). However, it remains poorly unknown whether there is a differential impact on frost ring formation depending on tree ontogeny and the age-related development of other tree organs, such as the bark. The hypothesis for this study is that bark thickness in *A. araucana* is age-dependent, linking this relationship to the probability that the derivative cells may be mostly exposed to different levels of frost damage when the trees are in their early stages of life. Therefore, if the increase in the bark thickness means more insulation, then the cambium may be more protected from frost as the tree ages. In this sense, a morphological traits such as the bark thickness could be an advantage to protect the vascular cambium from extreme colds during advanced stages of youth tree development, but may be inefficient during recruitment and development of new seedling generations, where plants have not yet formed an efficient bark isolation (Hantemirov et al., 2000; Gu et al., 2008). These considerations may be relevant topics in the ecology of plant communities under a global warming scenario, where more frequent and intense climate extremes, including freezing events, are expected.

2. Materials and methods

2.1. Study area

The study sites are located at the northern distribution area of the *A. araucana* forests, characterized by open woodlands intermingled by the Patagonian steppe (Golluscio et al., 1982; Schlichter and Laclau, 1998). Soils receive 500 mm of precipitation per year and the mean annual temperature is 12.4 °C. Winter months have a uniform atmospheric circulation originated from the Pacific, whereas summer has a relatively weak zonal component superimposed on the meridional gradient. Therefore, the west winds have a southerly component (west–southwest to southwest). These winds are characterized not only by their prevalence during the entire year but also by their higher seasonal speeds, particularly from October to February (summer) (Prohaska, 1976).

Temperature in our study region is the highest in Patagonia at a continental level, with harsh winters and temperate summers (Rubí Bianchi and Cravero, 2010). The relative humidity decreases from the Andes to the steppe, which causes an increase in the daily thermal amplitude in the same direction. Both conditions enhance the likelihood of frost occurrence (Bustos, 2001). This area experiences a mean annual frost-free period of 90 days (Movia et al., 1982), with December 1st as the date of the last late frost and March 21st as the date of the first early frost (Bustos, 2001). This period of frost risk, either corresponding to early or late frosts, concurs with the period of active division of the cambial cells and with the consequent growth ring formation in *A. araucana*.

The sites considered in this study represent two different topographical conditions: one is the Primeros Pinos (PP) site located in a plateau east from the mountain foothills and the second one is the Picún Leufú (PL) site located on the SE slope of the foothills of the Andes, which is more protected from western winds (Fig. 1).

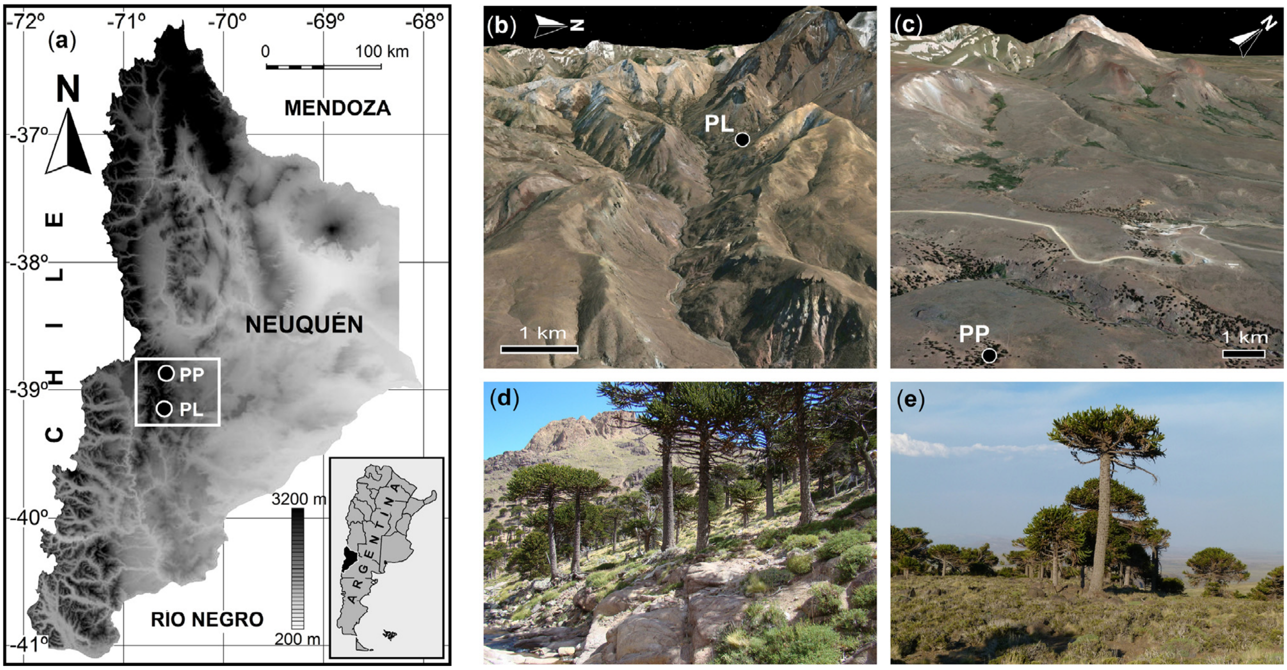

Fig. 1. Location of study sites in northern Patagonia. (a) Province of Neuquén, showing the sample sites. (b) PL site (1670 masl, 39°08′44.8″S–70°35′33.2″W). (c) PP site (1680 masl, 38°52′0″S–70°34′27″W). (d) The PL is a site where the forest grows on a southeast facing steep slope. (e) The forest at the PP site is placed on a high plateau.

2.2. Fieldwork

Wood samples were collected with an increment borer (Ø 5 mm) from the main stems of 30 live *A. araucana* trees per site. Trees were selected with stem diameters between 15 and 40 cm at breast height, which approximately correspond to trees of ≤200-yrs of age (Hadad, 2013). In order to reduce the influence of forest microclimate, as may occur in closed forests (Gurskaya and Shiyatov, 2006), trees sampled were separated by a minimum of 3 m distance from each other. Wood samples were taken from stems at two positions above ground: 0.50 m and 1.50 m. At each height, stem diameter was measured and four samples were collected, each corresponding to the cardinal directions (N, S, E, W) to assess whether the prevalence of the west–southwest and southwest winds during summer have a differential effect in the occurrence of frost damage to the vascular cambium depending on the orientation of the stems. In this sense, a total of eight cores were taken per tree. Cardinal directions were also used to standardize the sampling method reducing errors due to potential circumference anomalies. At the time of sampling, thickness of bark was measured in each of the collected woods. A total of 480 wood samples were collected for this study.

2.3. Laboratory work

Wood samples were mounted on wooden supports and delicately polished to attain a perfect anatomical identification of the boundary between growth rings. Afterwards, growth rings were visually dated following traditional dendrochronological protocols (Stokes and Smiley, 1968). Ring widths were measured using a measuring device (Velmex, USA) with a precision of 0.001 mm. Statistical quality control of the ring-width measurements was performed using the COFECHA program (Holmes, 1983). Samples with dating problems were rejected, reaching a final sampling of 197 series from 25 trees at the PP site and 223 series from 28 trees at the PL site.

Frost rings were anatomically identified during the sample dating procedure. Every growth ring showing a frost injury was marked as a frost ring. Each frost ring was distinguished according to the distance of sampling from the ground, the cardinal orientation and the inner position of the frost injury in the growth ring. In relation to the last observation, and based on Stöckli and Schweingruber (1996), frost rings were classified into three types: (1) frost injury at the beginning of the earlywood (EWF), (2) frost injury at the middle of the earlywood (MEWF), and (3) frost injury in the transition between the early and the latewood portion (LWF). The position of the frost injury within the growth ring indicates if the frost damage was due to a late or an early frost event during the growing season.

2.4. Statistical analysis

The cambial age of the trees (rings from the pith) was determined at each stem sampling position relative to ground level. For those series not reaching the pith, but with the inner arch close to it, the number of missing rings was estimated following the geometric method proposed by Duncan (1989). Considering the four cores per height, ordering the frost ring records from the first cambial year and counting the number of frost rings per year, we obtained the number of frost rings in relation to the cambial age. However, in order to avoid an overestimation of the number of frost rings, only one frost ring per cambial age and per tree was considered when relating the presence of frost rings with cambial age. The Pearson correlation was used to correlate the thickness of the bark with cambial age. Furthermore, to compare the frequency of frost rings in relation to bark thickness in the same period of tree life, both variables were analyzed at 30 year intervals. At each age class the mean and standard deviation of both variables were calculated and a trend line was fitted. Then, a regression analysis was done to test the relationship between the probability of frost rings and the bark thickness at different age classes. On the other hand, it was determined the percentage of frost rings in relation to the total number of growth rings analyzed, the percentage of growth rings with double frost injuries, and the percentage of EWF, MEWF and LWF rings in relation to the total frost rings recorded. A Logarithmic Linear Model (MASS library of R: GLM, formula = NUM ∼ SITE + TYPE,

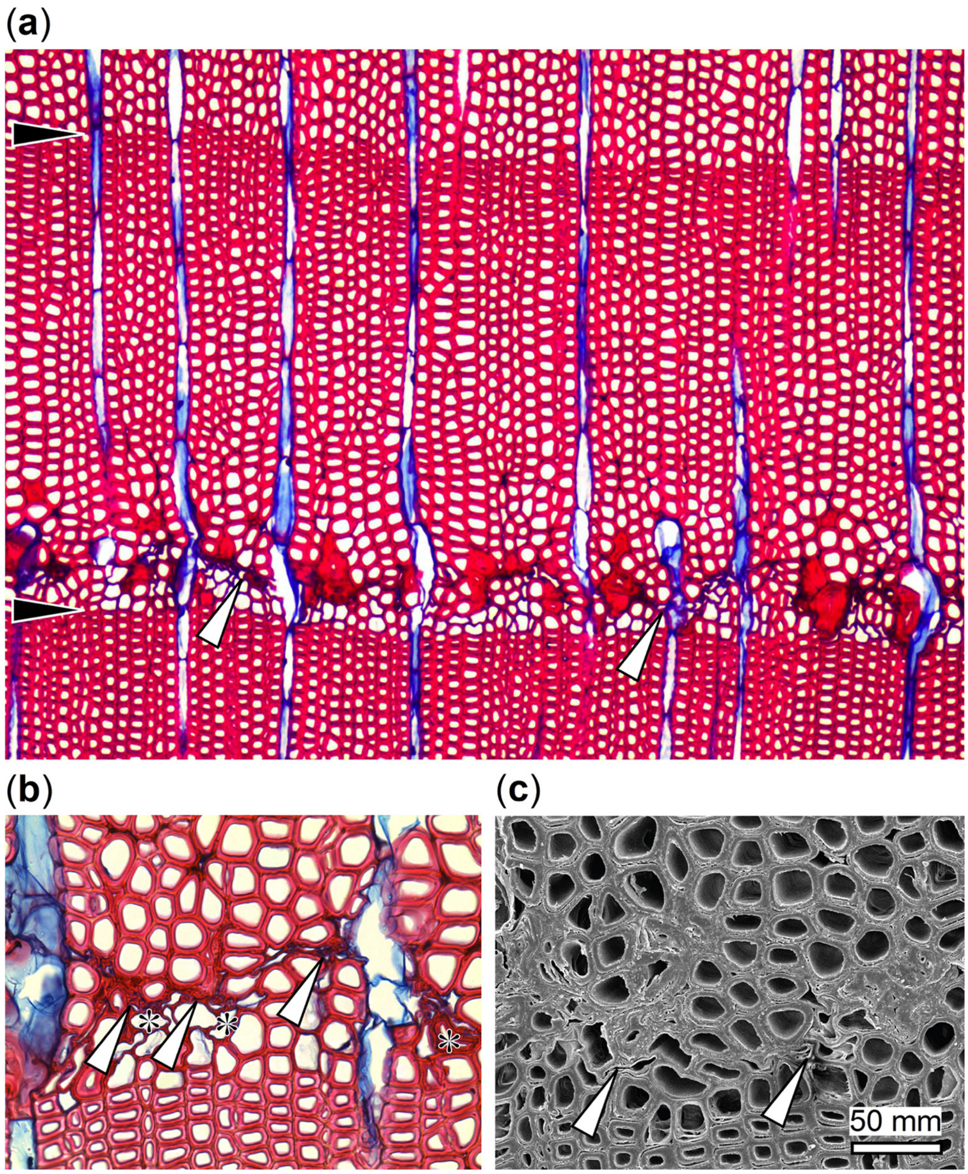

Fig. 2. Frost injuries in the xylem of *Araucaria araucana* trees growing at the northern natural geographical range of the species. (a) A general view of a frost injury caused by a late frost event, identified at the middle of the earlywood and composed by deformed, collapsed and distended rays that are offset at the zone of the injury (white arrows) (4×). Black arrows show the limit of the growth ring. (b) Detail of collapsed and deformed tracheid cells (asterisks and arrows) (20×). (c) A SEM image showing the traumatic frost region with some tracheids greatly collapsed and extremely thin-walled (arrows) among thick-walled and less collapsed tracheids.

data = loglinear) was used to test whether the type of structure of frost rings varies between the two analyzed sites.

The bark thickness and the presence of frost rings were analyzed according to both sampling distances from the ground and cardinal orientations. Statistics were performed by considering the period of time shared by the eight cores of each tree. A Linear Mixed Model (LMM) and a Generalized Linear Mixed Model (GLMM) were employed to analyze the bark thickness and the presence of frost rings, respectively. Those models are a valuable methodology to analyze complex data such as that contained in this paper. The sampling involved many sources of variability. Therefore, the LMM and the GLMM included factors with fixed and random effects. In this manner, the real effect of a causal factor that affects a process was determined, avoiding irrelevant sources of variability (Venables and Ripley, 2002; Zuur et al., 2009). For the analysis of frost ring presence, a binomial family with a logit link function was specified in the GLMM, that predicted the probability of frost rings in the total growth rings analyzed at each core.

In this sense, the conceptual model for frost ring presence was:

$$\mathbf{Frostringpresence} \sim \mathrm{Fixedeffects(Orientation + Height)} + \mathrm{Randomeffects(Intra\text{-}treevariabilityinsideofsitevariability)}.$$

For the bark thickness, a similar model was developed. Under the R notation and by using the library nlme (Pinheiro and Bates, 2000) for the LMM, and the library lme4 (Bates, 2010) for the GLMM, the syntax of both models was:

$$\mathbf{Frostringpresence} \sim \mathrm{Fixed : Orientation} + \mathrm{Height, Random : \sim Site|Tree}$$

$$\mathbf{Barkthickness} \sim \mathrm{Fixed : Orientation} + \mathrm{Height, Random : \sim Site|Tree}$$

Table 1
Number of analyzed tree rings, number of frost rings and number of types of frost rings according to sampling height in *Araucaria araucana* stems.

Stem height (m)	Tree rings	Primeros Pinos			Picún Leufú		
		Total tree rings	% of tree rings	% of frost rings	Total tree rings	% of tree rings	% of frost rings
0.5	Tree rings analyzed	10,134	100		13,827	100	
	Frost ring	482	4.76	100	106	0.77	100
	Double frost ring	12		2.49	0	0	0
	EWF frost ring	94		19.5	18		16.98
	MEWF frost ring	362		75.1	87		82.08
	LWF frost ring	14		2.9	1		0.94
1.5	Tree rings analyzed	8,001	100		11,466	100	
	Frost ring	327	4.09	100	94	0.82	100
	Double frost ring	11		3.36	0	0	0
	EWF frost ring	35		10.7	13		13.83
	MEWF frost ring	277		84.71	81		86.17
	LWF frost ring	4		1.22	0		0

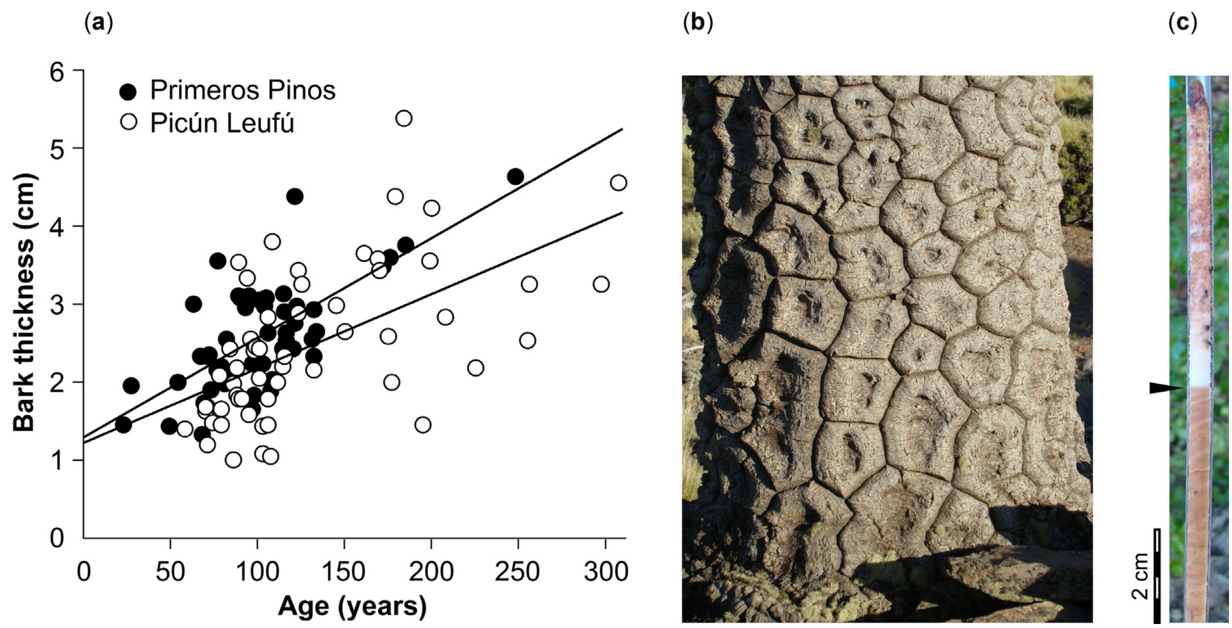

Fig. 3. (a) Variation of the bark thickness in *Araucaria araucana* according to tree age. Both sampled stem heights (0.5 m and 1.5 m) were considered for each tree at both PP and PL sites. (b) A tree stem showing a hard and tick bark organized in polyhedral-shaped plates. (c) An increment core showing the bark thickness. Black arrow indicates the position of the vascular cambium.

The significance of the different values of the models was determined by maximum likelihood analysis. All the statistics were referred to 5% of significance level.

3. Results

3.1. Identification and classification of frost rings

The frost injuries in the transversal xylem of the *A. araucana* trees were distinguished by a traumatic tissue composed by deformed and collapsed tracheids intermingled with locally distended and offset rays (Fig. 2). At both PP and PL sampled sites, we analyzed 43,428 growth rings (41.76% at PP and 58.24% at PL), and identified 1,009 frost rings. From these frost rings, 80.18% were recorded at PP and 19.82% at PL. Frost injuries exhibited different positions within the growth ring. We observed that MEWF and LWF rings occurred more and less frequently, respectively, independently of sites and stem position of the sample (Table 1). Sometimes, two successive frost injuries within the same growth ring appeared at the PP site. This particular structure was recognized as a double frost ring. The logarithmic linear analysis showed no significant differences in the type of structure of frost rings among sites (p-value = 0.29). The percentage of frost rings in relation to total tree rings analyzed was similar between the two sampling stem heights at both PP and PL sites (Table 1).

3.2. Relationship between bark thickness, frost ring probability and cambial age

When bark thickness was compared with cambial age, it was observed that bark width increased with age, both at PP (r = 0.69) and PL (r = 0.57) sites (Fig. 3). Furthermore, when analyzing the number of frost rings in relation to cambial age at both sites, it was clear that during the early period of the tree life the highest number of frost rings was recorded, while as cambial age increased the number of frost rings dropped progressively (Fig. 4). Even when frost rings were logged until about 150 years of the cambial age, 80% of total frost rings were identified during the first 45 and 70 years of growth at PP and PL, respectively. On the other hand, when analyzing the bark thickness and the frequency of frost rings at different age classes, it was observed that the bark thickness increased linearly with increasing age, while the frequency of frost rings decreased exponentially with cambial age, at least during the first

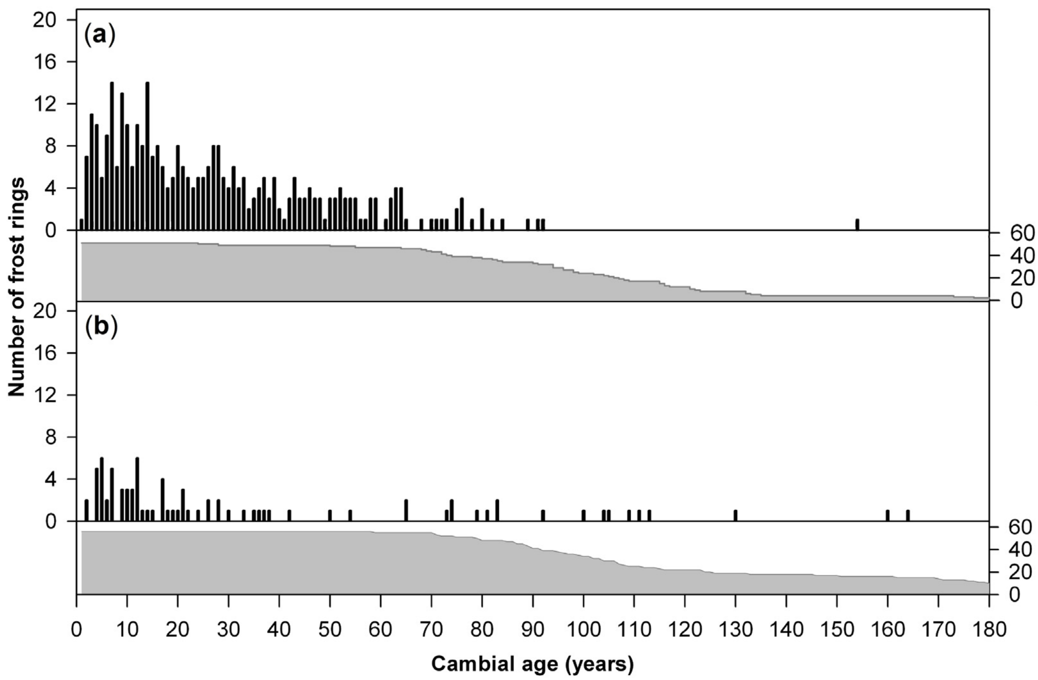

Fig. 4. Number of frost rings according to cambial age of *Araucaria araucana* at (a) PP and (b) PL. Gray areas indicate sampling depth.

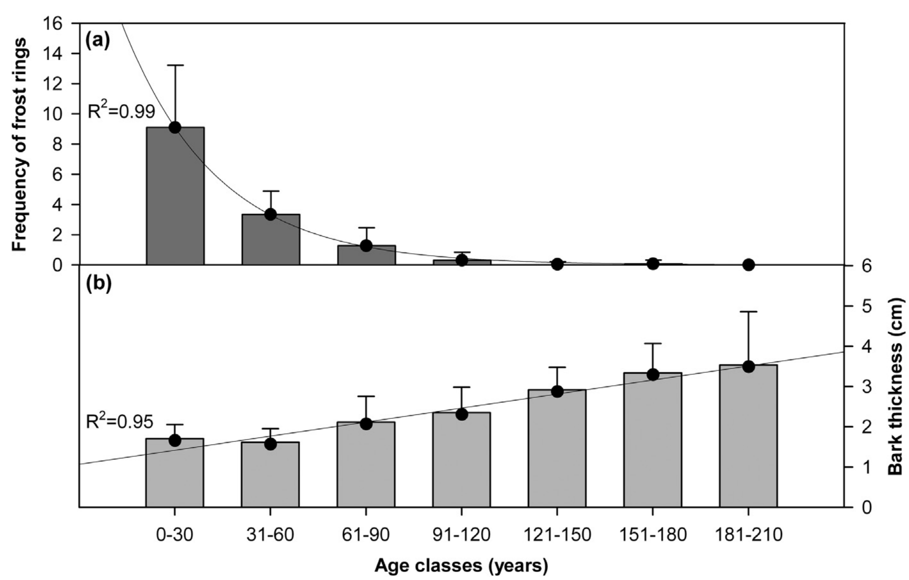

Fig. 5. (a) Mean of the frequency of frost rings and (b) mean of the bark thickness at different age classes in *A. araucana* trees. Bars indicate the standard deviation. A trend line is fitted in each case.

210 years of the tree's life (Fig. 5). Moreover, when the probability of frost rings and the bark thickness at different age classes were related, it resulted in a negative exponential relationship between these variables through the following regression function:

Probability of frost rings = 6.79 × exp (−2.51 × *Bark thickness*, cm) (Fig. 6)

3.3. Probability of frost rings and bark thickness

Results of both LMM and GLMM for bark thickness and probability of frost rings were statistically significant. In the case of frost ring probability, the sampling height showed a Chi-square-value of 36.17 (p-value < 1E-4) and the cardinal orientation a Chi-square-value of 9.55 (p-value = 0.0228). For bark thickness the F-value of sampling height was 218.44 (p-value < 1E-4) and for cardinal orientation the F-value was 5.61 (p-value = 9E-4). The coefficients of fixed effects for frost ring probability indicated that west and south sampling orientations have a positive effect on frost ring probability (Table 2). Moreover, sampling height position also showed a positive effect. In the case of the bark thickness, only height and south orientation were significant, showing a negative effect (Table 3).

4. Discussion

Prior reports indicate that *A. araucana* may record episodes of extreme cold in their wood, being a valuable proxy of past extreme temperatures in Patagonia (Hadad et al., 2012). Thus, a frost ring record allows not only to identify cold years but also to analyze

Table 2
Fixed effects for the Linear Mixed Model that relates bark thickness with cardinal orientation and sampling heights as fixed effects, and site and intra-tree variability as random effects.

Parameter	Coefficients	Standard error	*t*-value
Intercept	2.997	0.116	25.914***
North orientation	−0.031	0.073	−0.426 ns
West orientation	−0.083	0.073	−1.14 ns
South orientation	−0.272	0.073	−3.735***
Height: 1.5 m	−0.761	0.051	−14.78***

Significance levels: *0.05, **0.01, ***0.001.
No significant: ns.

Table 3
Fixed effects for the Generalized Linear Mixed Model that relates frost ring presence with cardinal orientation and sampling heights as fixed effects, and site and intra-tree variability as random effects.

Parameter	Coefficients	Standard error	*z*-value
Intercept	−5.889	0.949	−6.209***
North orientation	0.169	0.144	1.176 ns
West orientation	0.395	0.137	2.872**
South orientation	0.328	0.139	2.357*
Height: 1.5 m	0.593	0.098	6.024***

Significance levels: *0.05, **0.01, ***0.001.
No significant: ns.

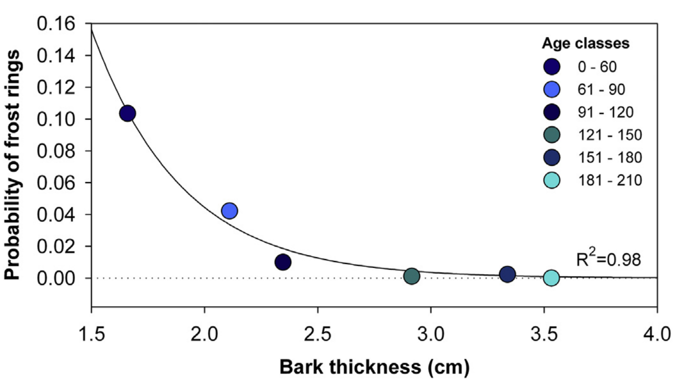

Fig. 6. An exponential regression curve fitted for the data of probability of frost rings and the bark thickness at different age classes (years) in *A. araucana* trees.

the frequency of these cold events, contributing to the discussion of the ecological implications of increasing weather extremes in the context of a warmer global climate (IPCC, 2007). However, although a frost record in wood is a useful tool to derive long-term extreme episodes in climate, many aspects related to the sensitivity of stems, including tree age and bark thickness as protective traits over meristems, still remain poorly documented. This in turn has an enormous implication to discern key ecophysiological consequences of freezing stress in forest ecosystem dynamics.

The formation of a frost ring may be related to diverse biophysical conditions, including intensity and duration of frost (Glerum, 1975; Stöckli and Schweingruber, 1996; Gurskaya, 2007), as well as topography at the stand level and forest structure (Geiger et al., 2003; Payette et al., 2010). In addition, and even when frost damage can be significant enough to lead to mortality of different plant tissues (Arco Molina et al., 2015), limits to frost resistance are variable and may depend on tree species, health, phenological stage, and the cryoprotectant function of the citoplasmatic cell density, among others (Pascale and Damario, 2004).

Frost damage in *A. araucana* wood was easily distinguishable as a traumatic ring area where the axial tracheids experienced the contraction (collapse) of its walls and the lost of their axial orientation. Moreover, rays appeared locally distended and deflected from the usual radial course of alignment when they were viewed in cross section. Even when the damaged area was characterized by such abnormalities of the tissue, the vascular cambium in *A. araucana* underwent a relatively rapid recovery on the production of new series of normal tracheids after the traumatic tissue during the same growing season (Fig. 2).

According to the observations of this study, the distribution of frost rings along the axis of the stem of *A. araucaria* showed a similar proportion in number at both 0.5 m and 1.5 m sampled points, independently of sites (Table 1). Gurskaya and Shiyatov (2006) studied the proportion of frost rings along the stems of *Larix sibirica* and *Picea obovata*, observing that the greater the distance from the ground the lower the frequency of the frost rings. The authors argued that during a freeze event the air masses are thermally stratified, with the colder layers remaining near the ground surface (Gurskaya and Shiyatov, 2006; Gurskaya, 2014). However, this thermal stratification can be modified by wind, mixing the layers of air with a consequent homogenization of the temperatures (Dy and Payette, 2007). A similar condition may be expected for our study sites, where strong and frequent winds, particularly between the spring-summer months of November and January, can mix air layers, preventing thermal stratification (Conti, 1998).

According to the results obtained, it could be speculated that as the bark of *A. araucana* becomes thicker, the thermal protection of the cambium in situations of extreme colds increases. This may be partially explained by the fact that trees with thicker bark could accumulate more heat along the day, so that the cambial zone remains warmer during a freeze. Under these conditions, frost-related damages in wood are rarely produced (Gurskaya and Shiyatov, 2006). Additionally, the sensitivity to frost varies in relation to the different parts of the tree stem and branches (Stöckli and Schweingruber, 1996; Schweingruber, 2007). When analyzing frost rings along the basal section of the trunk in *A. araucana* stems, it was observed that the probability of the vascular cambium to be damaged by frost is significantly lower at 0.5 m than at 1.5 m from the ground (Table 3). Coincidentally, the bark thickness was inversely related to the stem height, being significantly thicker at 0.5 m than at 1.5 m high from the ground level (Table 2). Moreover, when analyzing the frequency of frost rings and bark thickness at different age classes, it was observed that the frequency of frost rings decreased and bark thickness increased with increasing cambial age (Fig. 5), demonstrating an exponentially negative relationship between both variables (Fig. 6). These results suggested an inverse relation between the width of the bark and the probability of frost ring occurrence. On the other hand, when analyzing the presence of frost rings in relation to cardinal orientations, it was observed that the probability of frost ring was significantly higher in the south and west sides of the tree stems at both sites (Table 3). There are two possibilities to cause this result. First, the higher probability of frost ring in those sides of the stems concurred with the most frequent wind directions recorded for the study region, indicating that the cambium at that position may be more exposed to frost damage. A second cause is maybe related to the latitude where the studied trees are located. The north and east orientations are the warmest sides, which corresponds to the Southern Hemisphere. Thus, the north and east sides of the tree stems may accumulate more heat during the day and probably remain warmer than the south and west sides, thereby increasing the resistance of the cambium to damage by freezing temperatures. In this sense, any cause or the combination of both, may be responsible for the higher probability of frost rings in the south and west sides of the tree stems. In short, both the thickness of the bark and the orientation of the trunk can influence the degree of probability of which cambium can be damaged by an extreme cold.

The results of this study confirm the conjecture that as trees get older they gradually lose their capacity to record frosts in their wood structure (Fayle, 1981; Stöckli and Schweingruber, 1996; Treter and Block, 2004; Schweingruber, 2007; Payette et al., 2010). In *A. araucana* this could be partially explained by the direct relationship between bark thickness and tree age (Figs. 3 and 5), under the evidence that bark thickness is age-dependent. It was observed that these trees were particularly sensitive to be injured by frosts during the first 100 cambial years, a period corresponding to the juvenile stage of their life (Hadad et al., 2015). Gurskaya and Shiyatov (2006) indicated that a similar relationship was observed in *P. obovata* and *L. sibirica*, two species where bark thickness increased with tree age. By contrast, frost rings have been observed at any age of the tree life for species with a thin bark layer (Hantemirov et al., 2000). In *A. araucana* trees, 80% of frost rings was confined to the first 45 years of cambial age at the PP site and to the first 70 years of life in PL, although occasional records of frost rings were observed for up to 150 years of cambial age (Fig. 4). These occasional records might be the result of unusually intensive frosts that may affect both juvenile and older trees. In agreement with these results it was reported that 80% of the frost rings was recorded during the first 30 and 20 cambial years in *P. obovata* and *L. sibirica*, respectively (Gurskaya and Shiyatov, 2006). Moreover, Kidd and Copenheaver (2014) reported that 90% of the frost rings occurred between cambial ages 1 and 10 in *Pinus banksiana*, although tree age ranged from 13 to 104 years. Therefore, it appears that the sensitivity of *A. araucaria* to be damaged by frost decreases with increasing age, stem diameter and bark thickness (in agreement with the observations made by Glerum, 1975; Stöckli and Schweingruber, 1996; Gurskaya and Shiyatov, 2002; Treter and Block, 2004; Gurskaya, 2007; Waito et al., 2013; Kidd and Copenheaver, 2014), demonstrating that a higher probability of frost rings occurs at the juvenile portions of the xylem (as pointed out by LaMarche, 1970; Fayle, 1981; Brunstein, 1996; Gurskaya and Shiyatov, 2002; Payette et al., 2010).

Topography and other factors such as microclimate, canopy density and soil moisture, have been indicated as possible factors contributing to forest susceptibility to extreme cold events (Dy and Payette, 2007; Payette et al., 2010; Gurskaya, 2014; Kidd and Copenheaver, 2014). Differences in the frequency and probability of frost rings recorded at PP and PL sites, separated by 30 km, could be due to differences in topography. While the PL forest is located on a slope facing SE, and consequently relatively more protected from the westerlies, the trees on the PP site develop in a basaltic highland plateau, more exposed to the flow frequency and severity of these winds.

The position of the frost injury inside the growth ring may indicate the chance of occurrence of a frost during the period of the active plant growth development (Treter and Block, 2004; Payette et al., 2010). This makes it possible to distinguish if the climate event corresponds to a late (spring) or an early (autumn) frost (Treter and Block, 2004). According to the observations here reported, most of the frost rings in *A. araucana* are located at the earlywood zone, indicating a preponderance of late frosts (Table 1). These results agree with those reported by Hadad et al. (2012) from other *A. araucana* forest populations in northern Patagonia, a region where late frosts are frequent. Similar reactions of wood to late frost injuries have been observed in species from other regions of the world such as: *Pinus obovata*, *L. sibirica* (Gurskaya and Shiyatov, 2006), *Picea mariana* (Payette et al., 2010) and *P. banksiana* (Kidd and Copenheaver, 2014), suggesting a higher vulnerability of cambial cells during xylem differentiation to cold. Although northern Patagonia may experience early frosts (Bustos, 2001) with proven evidence of frost ring formation, no significant number of latewood frost rings were detected. This could be related to the low mean temperature in the last stage of the growing season, which leads to a lower rate of cambial activity and hence the mother xylem cells are rarely damaged by early frosts (Gurskaya, 2014). A similar fact has also been hypothesized for *P. mariana* (Payette et al., 2010). Particular growth rings with two frost injuries may indicate unusual cold summers, as claimed by Gurskaya and Shiyatov (2002). These rings, rare in *A. araucana*, were detected in the most exposed site to wind (PP site) and from other similar forest environments located 100 km far away from this site (Hadad et al., 2012).

5. Conclusions

In the present study the frost injuries were anatomically and dendrochronologically identified in the xylem of *A. araucana*, an endemic tree from northern Patagonia, being these injuries related to developmental traits. The presence and probability of frost rings, the bark thickness and the cambial age exhibited singular relationships, evidencing that bark thickness is age-dependent and that frost ring probability decreases as the thickness of bark and cambial age increase. Results showed a parallel increase in bark thickness and cambial age, and a decrease in the probability of frost rings. As shown by the physical evidence (frost rings and bark thickness) it is arguable that the bark has an insulating and protective capacity against the potential incidents of frost in the vascular cambium. However, physiological conditions associated with age and frequency of frost damage should not be discarded, a fact that should be studied in detail. Consequently, as 21st-century warming model scenarios predict that extreme cold weather events are still likely to occur over many regions of the world (Kodra et al., 2011), their impact over Patagonian forests and their possible consequences in forest dynamics should be addressed with particular attention.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.dendro.2016.01.003.

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