

# Vascular performance of woody plants in a temperate rain forest: lianas suffer higher levels of freeze–thaw embolism than associated trees

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## Summary

1. The success of lianas in tropical rain forests has been linked to their wide xylem vessels that confer much higher hydraulic conductivity than tropical trees. In contrast, the poorer representation of lianas in temperate forests and high elevations might reflect lower competitiveness there, because of the vulnerability of high-performance xylem to freeze–thaw embolism. Nevertheless, the empirical evidence that support this idea is surprisingly scarce.

2. We assessed this hypothesis comparing the hydraulic performance of five lianas and nine trees coexisting in cold weather, by measuring wood traits, hydraulic conductivity, xylem embolism and root pressures.

3. Vessel diameters of lianas were on average twice those of trees (51.3 vs. 23.2  $\mu\text{m}$ ), resulting in an 18-fold difference in mean specific hydraulic conductivity (15.5 vs. 1.05  $\text{kg s}^{-1} \text{m}^{-1} \text{MPa}^{-1}$ ). Lianas on average suffered higher levels of freeze–thaw embolism than trees (29.9 vs. 12.8%), reducing their potential hydraulically supported competitive advantage; however, partially embolized lianas still had greater water transport capacity than trees.

4. Specific hydraulic conductivity ( $K_s$ ) was well correlated with vessel size in lianas but not in trees; however, plants with big vessels were more vulnerable to freeze embolism. Although all species of lianas developed root pressures and these tended to be higher than trees, root pressure were insufficient to repair embolism in plants  $> c. 7$  m tall. The magnitudes of root pressure were well correlated with vessel size in lianas, but this relationship was not observed in trees.

5. *Synthesis*: Our results show empirical evidence that hydraulic performance of temperate lianas exceed the performance of coexisting trees, in similar magnitude than its tropical counterparts. Nevertheless, this hydraulic advantage is reduced as a consequence of freeze embolism. Temperate lianas appear to employ a bet-hedging strategy entailing significant embolism in winter, but likely enabling rapid carbon gain during frost-free periods. Loss of winter carbon gain opportunities because of embolism may explain the low success of lianas in temperate rain forest. However, predicted future climatic scenarios could be likely to increase liana abundance in mid-latitudes and allow lianas to expand their ranges towards higher latitudes and elevations.

**Key-words:** liana distribution, root pressure, specific hydraulic conductivity, vessel diameter, woody vines

## Introduction

Lianas (woody vines) have thin, flexible stems, incapable of growing erect as adults. Therefore, they require mechan-

ical support of other plants to reach the canopy in forests (Darwin 1867; Schenck 1892, 1893; Isnard & Silk 2009), and so are considered structural parasites (Putz 1984; Stevens 1987).

Nowhere else are lianas as well-represented in woody floras as in the tropics (Gentry 1991; Phillips & Miller 2002; Schnitzer & Bongers 2002; Hu, Li & Li 2010). Lianas

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represent on average about 25% of woody plant species in tropical forests, falling to *c.* 10% in temperate forests (Gentry 1991). Similarly, both liana richness and their proportional representation in woody floras decline with increasing elevation, usually disappearing completely well below tree line (Vazquez & Givnish 1998; Jiménez-Castillo, Wisser & Lusk 2007). These general patterns of liana distribution suggest that this life-form is less viable in cold environments (Gentry 1991; Ewers, Cochard & Tyree 1997; Schnitzer & Bongers 2002; Hu, Li & Li 2010).

In the tropics, a highly efficient vascular system is probably one of the key adaptations enabling lianas to scale and overgrow host trees (Ewers & Fisher 1991; Holbrook & Putz 1996; Schnitzer 2005). Xylem vessels of tropical lianas are at least twofold wider than those of tropical trees (Gartner *et al.* 1990; Ewers, Fisher & Fishner 1991), and the possession of such vessels enables these plants to supply water to a large leaf area with a small cross-sectional area of xylem (Schenck 1893; Ewers 1985; Gartner *et al.* 1990; Ewers & Fisher 1991). According to the Hagen–Poiseuille law, describing the flow of water through ideal capillaries, hydraulic conductance of a vessel (water flow divided by pressure;  $k = \text{m}^3 \text{s}^{-1} \text{MPa}^{-1}$ ) is proportional to the fourth power of its radius ( $k \propto r^4$ ; Zimmermann 1983; Ewers & Fisher 1989), so a twofold difference in vessel diameter (or radius) could imply a 16-fold difference in conductance. Thus, by producing hydraulically efficient xylem, which has little role in support, lianas are able to allocate much less biomass to stemwood than other woody plants (Putz 1983; Gartner *et al.* 1990; Ewers & Fisher 1991), permitting more investment in roots and leaves, and hence faster growth (Holbrook & Putz 1996; Schnitzer & Bongers 2002; Restom & Nepstad 2004; Schnitzer 2005).

In temperate evergreen forests, frequent frosts would be expected to erode the competitiveness of lianas as a group. Wide conduits are susceptible to freeze–thaw embolism (Davis, Sperry & Hacke 1999; Pate & Canny 1999; Field & Brodribb 2001), which occurs when gas bubbles formed when xylem sap freezes fail to re-dissolve on thawing, cutting the water column in the conduit and reducing water flux to the leaves (Tibbetts & Ewers 2000). Impairment of the function of wide vessels in frosty climates could seriously limit the ability of liana vascular systems to supply leaves with water, compromising their growth rates and ability to outcompete other woody plants (Sperry *et al.* 1987; Ewers, Fisher & Fishner 1991; Ewers, Cochard & Tyree 1997). The relatively thin and hence poorly insulated stems of lianas might heighten their vulnerability to freezing temperatures.

How, then, do lianas respond to the dilemma of temperate maritime climates, where freezing conditions are unpredictably interspersed with carbon gain opportunities throughout much of the year? This type of climate might select for smaller vessels that are less susceptible to freeze–thaw embolism, resulting in a safer vascular system but lower conductivity and growth. Alternatively, large-diameter vessels might still be favoured if the positive impact of carbon gain during frost-free periods outweighs the cost of recurrent embolism

during the colder months. Or are temperate rain forest lianas able to quickly flush out embolism by developing positive root pressures (Sperry *et al.* 1987; Tibbetts & Ewers 2000)? Each of these potential responses implies some sort of cost likely to result in lianas being less competitive in maritime temperate climates than in the tropics. However, there are few data on the comparative structure and function of the vascular system of lianas and trees in temperate rain forests.

In this study, we explore the anatomy and function of the vascular systems of lianas and co-occurring trees in a Chilean evergreen temperate rain forest. We addressed the following questions: (i) Is the vascular system of temperate rain forest lianas hydraulically more efficient than those of associated trees? (ii) Is this advantage reduced by differential susceptibility to freeze–thaw embolism? (iii) Do temperate lianas generate high root pressures that would serve to quickly revert embolism? We measured vessel diameters and densities, specific hydraulic conductivity ( $K_s$ ) and freeze–thaw embolism as percentage of loss conductivity (PLC) in branches of five species of lianas and nine of trees. We also measured root pressure and used the Hagen–Poiseuille law to estimate the relative contribution of different vessel size classes to the overall hydraulic conductivity of stems of each species.

## Materials and methods

### STUDY AREA

Samples were collected during winter in an evergreen temperate rain forest located on the south-eastern slopes of the Cordillera de Nahuelbuta (37°41'S, 73°12'W; 750 m a.s.l.), in south-central Chile (Jiménez-Castillo, Wisser & Lusk 2007). We chose to work at 750 m a.s.l. because five liana species reach this elevation, with liana species richness declining rapidly further upslope. The climate is maritime temperate, with frequent sub-zero temperatures during winter and spring (di Castri & Hajek 1976). During the 16-day sampling period, we recorded daily maximum and minimum temperatures using a max/min thermometer (Supporting Information, Table S1). Annual precipitation is *c.* 2000 mm near the coast, rising to *c.* 3000 mm near the range summits, with frequent snowfalls during winter above 800 m a.s.l. (Almeida & Sáez 1958; di Castri & Hajek 1976). The vegetation is mature Valdivian rain forest (Veblen, Schlegel & Oltremari 1983), dominated by the tree species *Aextoxicon punctatum* R. et P. (Aextoxicaceae), *Eucryphia cordifolia* Cav. (Cunoniaceae), *Nothofagus dombeyi* Mirb. Oerst (Fagaceae), *Gevuina avellana* Mol. (Proteaceae) and *Luma apiculata* (DC) Burret (Myrtaceae). Less abundant are *Amomyrtus luma* Mol. (Myrtaceae), *Persea lingue* (Lauraceae), *Dasyphyllum diacanthoides* (Less.) Cabr. (Compositae) and *Weinmannia trichosperma* Cav. (Cunoniaceae). The forest canopy averages about 30 m in height, with emergent *N. dombeyi* attaining *c.* 40 m. All five liana species frequently reach the canopy. The study area has most of the lianas described from the region, including *Berberidopsis coralina* Hook. f. (Berberidopsidaceae), *Boquila trifoliolata* (Dc.) Dcne (Lardizabalaceae), *Cissus striata* R. et P. (Vitaceae), *Hydrangea serratifolia* (H. et A.) F. Phil. (Hydrangeaceae) and *Elytropus chilensis* (A.DC.) Muell. Arg. (Apocynaceae). Two other liana species found in the area, *Lardizabala bieternta* R. et P. (Lardizabalaceae) and *Campsidium valdivianum* (Phil.) Skottsb. (Bignoniaceae), were not considered due to their local scarcity. All liana and tree species were evergreen.

## SPECIFIC HYDRAULIC CONDUCTIVITY

The most common measures of hydraulic transport efficiency do not always allow comparisons between species or individuals. For example, *hydraulic conductance* (water flux divided by pressure;  $k = \text{kg s}^{-1} \text{MPa}^{-1}$ ) and *hydraulic conductivity* (water flux through a unit length of stem divided by pressure gradient;  $K_h = \text{kg m s}^{-1} \text{MPa}^{-1}$ ) do not consider the cross-sectional area of active xylem. For our purposes, we considered it appropriate to measure the specific hydraulic conductivity ( $K_s$ ), corresponding to  $K_h$  per unit cross-sectional area of the xylem ( $A_x$ ). This measure normalizes the flow of water through the xylem by length unit and by xylem transversal area unit, making the hydraulic transport measures between different-sized segments comparable:

$$K_s = FL/(\Delta P A_x) \quad \text{eqn 1}$$

$$K_s = \text{kg s}^{-1} \text{m}^{-1} \text{MPa}^{-1}$$

where  $F$  is the water flow mobilized through the segment expressed in mass ( $\text{kg s}^{-1}$ ) or volume ( $\text{m}^3 \text{s}^{-1}$ ),  $L$  is the length of the segment (m),  $\Delta P$  is the pressure difference between the ends of the segment ( $\text{MPa}^{-1}$ ) and  $A_x$  is the xylem transversal area ( $\text{m}^{-2}$ ).

$K_s$  measurements were taken on branches from five individuals of each species. Branches were 5–15 mm in diameter and exposed to full sunlight for at least part of the day. Samples were cut in pre-dawn, between 1.0 and 1.5 m above-ground. Immediately after cutting, branches were re-cut under water about 0.5 m higher. After being transported to base camp, located 15 min from the sampling site, branches were cut again c. 0.2 m higher under water. Care was taken that the segments used for the measurements were free of side branches and were at least as long as the maximum conduit length found in the species in question. This was to prevent incorrect measurement of  $K_s$  caused by high water flow through open vessels, or by the immediate flushing of embolisms out of open vessels. The maximum length of the vessel was estimated by injecting pressurized air (c. 60 kPa, see Ewers & Fisher 1989) to the proximal end of the fresh branches of at least 2 m length. The distal end was submerged in water and repeatedly cut in 1 cm sections until bubbling was visible, indicating breaching of the longest vessel. A maximum length value was obtained averaging branches of five individuals per species, ranging from 26 to 35 cm for lianas and 12–22 cm for trees.

$K_s$  was measured using a field system based on a pressure drop flowmeter (See detailed description in Tyree *et al.* 1993, 1994; Brodribb & Field 2000). Once  $k$  of the sample is known via flowmeter,  $K_s$  is calculated as indicated above (eqn 1), standardizing by unit of length of the segment and area of the hydraulically active xylem.

The fluid used was a KCl solution (10 mM), prepared with de-ionized water and filtered to eliminate particles of  $>0.2 \mu\text{m}$ . This solution is used to avoid any reduction in conductivity in the sample, which may happen using pure water (Sperry *et al.* 1987; Zwieniecki, Melcher & Holbrook 2001). Cross-sectional area of the conducting xylem was calculated after  $K_h$  was measured in a sample. Safranin dye (0.1%) was forced through the segment, which was then sectioned transversally. Next, the xylem conductor transversal area was calculated averaging the stained area of the two ends of the segment. The area of the pith and any unstained xylem in the interior were subtracted from the total area.

## LOSS OF CONDUCTIVITY DUE TO EMBOLISM

We estimated the percentage of loss conductivity (PLC) caused by embolism. Initially, we measured  $K_s$  as described before, obtaining

the winter-time field  $K_s$  of the sample. Then, the potential embolism in the sample was flushed out by refilling the segment with KCl solution at a pressure of  $\approx 170$  kPa for 10–15 min (Sperry *et al.* 1987), and a new measurement of  $K_s$  taken before calculating the stem cross-sectional area. This last value of  $K_s$  was taken to represent the maximum water transport capacity of the branch segment. Then, PLC is calculated as  $\text{max } K_{s\text{-field}} K_s / \text{max } K_s$ . All samples were collected in the last 2 weeks of winter in pre-dawn conditions.

## VESSEL DIAMETER AND DENSITY

After all field measurements were taken, a piece of each segment was labelled, stored in a plastic bag and taken to the laboratory to measure vessel diameter and density. Freehand xylem transverse sections were cut with a surgical blade and viewed under a light microscope (Nikon, Tokyo, Japan) at  $100\times$  magnification. Sections were photographed with a digital camera (Nikon Coolpix 4300, Nikon). Each transverse section was divided into four sectors, and vessel density and diameters were recorded in a continuous transect from pith and bark within each sector. The diameter of each vessel was calculated averaging the longest and shortest axis across the lumen (Ewers & Fisher 1991), using SIGMASCAN PRO 5 (SPSS Inc). An average was calculated for each branch ( $n > 100$  vessels) and an average for each species (five branches). Density was measured as number of vessels per unit of area tissue in stem transversal section. Because the liana *B. trifoliolata* has wide rays, we took care to not include rays in vessel density estimation for this species, in an attempt to obtain values comparable with those of the other species.

Vessels were grouped in  $10\text{-}\mu\text{m}$ -diameter classes, and the relative contribution of each class to total hydraulic conductivity was calculated using the Hagen–Poiseuille law (Gorsuch, Oberbauer & Fisher 2001). The contribution of each vessel diameter class was calculated as the sum of the fourth powers of all vessel radii in the class divided by the sum of the fourth powers of all vessel radii.

## ROOT PRESSURE

Root pressure was estimated with bubble manometers on five individuals of each liana and tree species studied, as described by Ewers, Cochard & Tyree (1997). During the night prior to measurement, one lateral branch was cut and a manometer attached with a tight-fitting vinyl tube. Each manometer consist in a glass tube of 1.0 mm internal diameter closed at the distal end, filled with distilled water in basal half and air bubble in the distal. The length of bubble was measured in pre-dawn ( $L_{pd}$ , in m) and immediately the vinyl tubing was cut and the bubble length measured again at atmospheric pressure ( $L_{atm}$ , in m). The root pressure ( $P_R$ , in kPa) was estimated using the eqn 2, derived from of ideal gas law (Ewers, Cochard & Tyree 1997; Tibbetts & Ewers 2000).

$$P_R = 100[(L_{atm}/L_{pd}) - 1] \quad \text{eqn 2}$$

The critical pressure for reverting embolism ( $P_c$ , in kPa) was calculated using eqn 3, from methodology proposed by Ewers, Cochard & Tyree (1997), where  $t$  is the surface tension of water and  $D$  the vessel diameter.

$$P_c = -4t/D \quad \text{eqn 3}$$

Because roots push up water against a gravitational gradient of  $y - 10 \text{ kPa m}^{-1}$ , the maximum height for reverting embolism ( $h_c$ ) by a given root pressure near the stem base ( $P_{RB}$ , in kPa) (Yang & Tyree 1992; Lewis, Hamden & Tyree 1994) is given as follows:

$$h_c = (P_{Rb} - P_c)/10 \quad \text{eqn 4}$$

STATISTICA (StatSoft Inc., Tulsa, OK, USA) was used to carry out nested ANOVA testing for differences in mean vessel diameter, mean vessel density,  $K_s$  and PLC between liana and tree life-form, as well as differences among species within each life-form. Relationships between vessel diameter– $K_s$  and vessel diameter–vessel density were explored using the software JMP4 (SAS Institute Inc., Cary, NC, USA.)

## Results

Vessels were on average about twice as wide in lianas (mean, 51.3  $\mu\text{m}$ ) as in trees (mean, 23.2  $\mu\text{m}$ ) (Table 1). Mean vessel diameter of liana species varied from 36  $\mu\text{m}$  in *Elytropus chilensis* to 65  $\mu\text{m}$  *Cissus striata*, while in trees mean diameters ranged from 18  $\mu\text{m}$  in *Gevuina avellana* to 36  $\mu\text{m}$  in *Persea lingue* (Fig. 1a). The xylem of lianas also included a wider range of vessel diameters than tree xylem; coefficients of variation ranged from 10.8 to 30.7 among lianas, compared with 7.3 to 18.7 in trees. Some liana species had vessels of up to 190  $\mu\text{m}$  diameter. Within each life-form, mean vessel diameter differed significantly among species (Table 1).

Vessel density was higher on average in trees than in lianas, and there were significant differences among species within each life-form (Table 1). As expected, vessel density was negatively correlated with vessel size in trees, while in lianas, this relationship was marginal (Fig. 2b).

The efficiency of hydraulic transport was much higher in lianas, exceeding that of trees by about 18-fold on average (Table 1).  $K_s$  ranged between 8 and 30  $\text{kg s}^{-1} \text{m}^{-1} \text{MPa}^{-1}$  in lianas, but did not exceed 3.5  $\text{kg s}^{-1} \text{m}^{-1} \text{MPa}^{-1}$  in trees it (Fig. 1b). There was significant interspecific variation within both groups (Table 1). As expected, lianas showed a positive correlation between  $K_s$  and vessel diameter, but surprisingly this relation was negative in trees (Fig. 2a). There was no relationship between  $K_s$  and vessel density in lianas or trees.

A few large vessels were responsible for most water transport in lianas (Fig. 3). Although vessels >100  $\mu\text{m}$  diameter comprised only 2.2–3.1% of all liana vessels, they accounted for 23–31% of the total hydraulic conductivity (Fig. 3). The most extreme case was *C. striata*, in which the largest 6.5% of vessels (140–190  $\mu\text{m}$ ) were responsible

for almost 50% of the total hydraulic conductivity. In contrast, the modal diameter class in most lianas (30–40  $\mu\text{m}$ ) concentrated 25–53% of the vessels, but contributed only 0.7–38% of the total hydraulic conductivity.

Tree vessel diameters did not exceed 80  $\mu\text{m}$ . In eight of nine species, the highest vessel frequency was recorded between 10 and 30  $\mu\text{m}$  diameter (57–87%), concentrating 39–64% of the total hydraulic conductivity (Fig. 4). In trees, although the largest diameter classes also conducted more than the smaller vessels, the modest range of vessel diameters meant there was less disproportion between the relative abundance of different diameter classes and their contributions to hydraulic conductivity.

Lianas suffered higher average levels of embolism in winter (29.9%) than trees (12.8%). Loss of conductivity ranged from 10.6 to 76.6% in lianas, compared with 0.29–23% in trees (Fig. 5); notwithstanding this wide interspecific variation within both life-forms, trees and lianas differed significantly in average loss of conductivity (Table 1). Across the 13 species of trees and lianas, percentage loss of conductivity was positively correlated with mean vessel diameter (Fig. 6a).

We detected root pressures in 13 of 14 species studied (Table 2). Although root pressures of lianas averaged nearly twice those of trees (30.5 v/s 16.6 kPa), this difference was no significant because of wide interspecific variation within both life-form. The highest root pressures recorded for lianas and trees could to repair embolism to heights of 7.1 and 8.1 m, respectively (Table 2). Root pressures of lianas were strongly positively correlated with vessel diameter, but not those of trees (Fig. 6b).

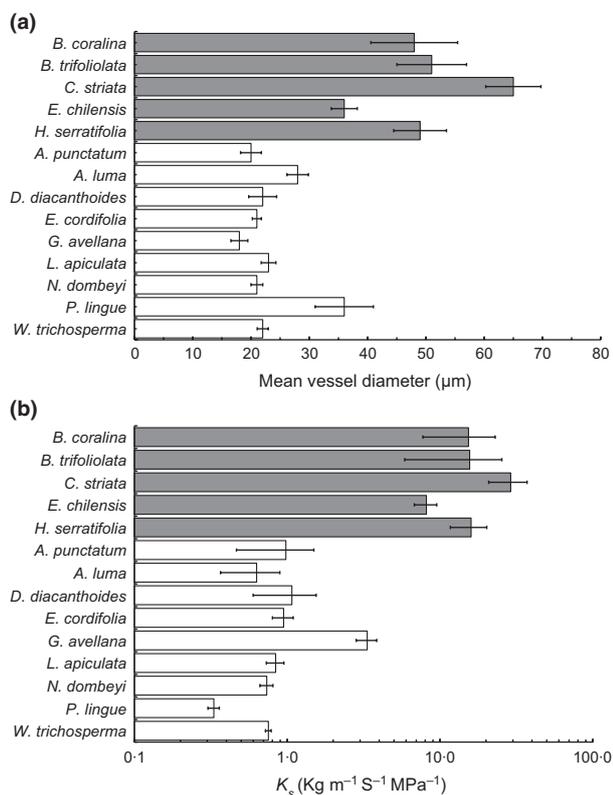
## Discussion

### HYDRAULIC PERFORMANCE

Water transport in lianas studied was found to be much more efficient on average than that of associated tree species (Table 1), reflecting the larger vessel sizes of the former. The 18-fold difference in average conductivity is within the range of previous differences reported from tropical forests in Mexico and Panamá, where lianas were respectively 24 and 11 times more efficient than co-occurring trees (Gartner *et al.* 1990; Patiño, Tyree & Herre 1995). This suggests that,

**Table 1.** Mean of xylem traits ( $\pm$ SE) for liana ( $n = 5$ ) and tree life-form ( $n = 9$ ) in a temperate rain forest, and results of nested ANOVA comparing the two life-forms and species inside of each life-form. Number of samples for each specie ranged between 4 and 5

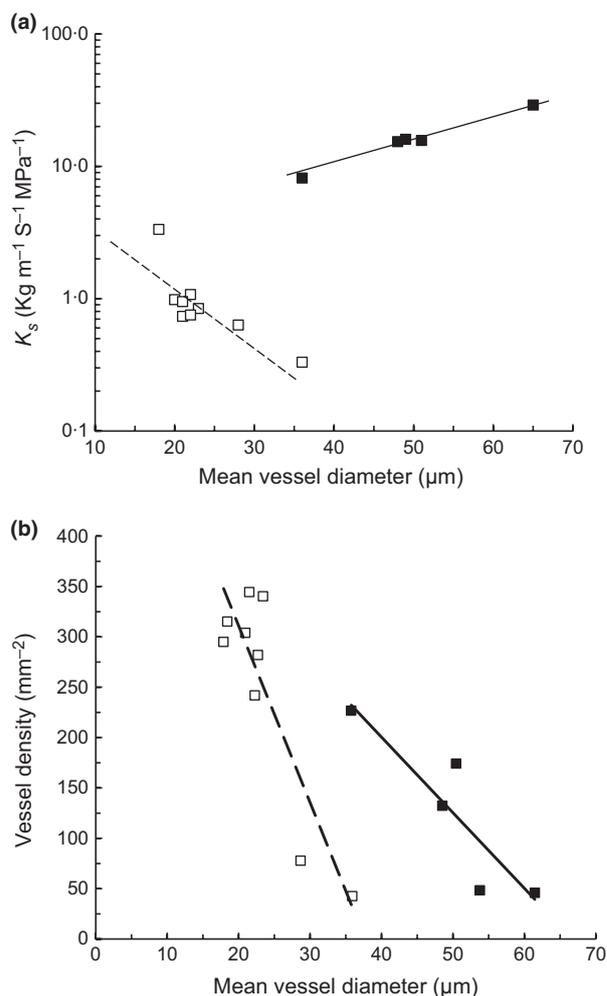
Variable	Lianas	Trees	Effect	d.f.	<i>F</i>	<i>P</i>
Mean vessel diameter ( $\mu\text{m}$ )	51.3 $\pm$ 2.7	23.2 $\pm$ 1.0	Life form	1	179.1	<0.00001
			Species (Life form)	12	3.22	0.0032
Mean vessel density ( $\text{mm}^{-2}$ )	122.8 $\pm$ 18.4	252.4 $\pm$ 23.7	Life form	1	37.8	<0.00001
			Species (Life form)	12	7.1	<0.00001
Mean $K_s$ ( $\text{kg s}^{-1} \text{m}^{-1} \text{MPa}^{-1}$ )	20.2 $\pm$ 3.6	1.1 $\pm$ 0.14	Life form	1	386.6	<0.00001
			Species (Life form)	12	4.22	0.0003
Mean PLC (%)	29.9 $\pm$ 5.1	12.8 $\pm$ 2.4	Life form	1	50.9	<0.00001
			Species (Life form)	12	8.8	<0.00001



**Fig. 1.** (a) Vessel diameter and (b) specific hydraulic conductivity ( $K_s$ ) in lianas (grey bars) and trees (white bars) in Cordillera Nahuelbuta (mean  $\pm$  1 SE).

during frost-free periods, lianas of temperate forests will have potentially similar competitive advantages to lianas in the tropics. However, at Nahuelbuta, both life-forms had lower transport capacity than their respective tropical counterparts, reflecting smaller vessel diameters at our temperate rain forest site. The average  $K_s$  of Chilean lianas ( $20.2 \text{ kg s}^{-1} \text{ m}^{-1} \text{ MPa}^{-1}$ ) was less than the equivalent mean figure found by Ewers, Fisher & Fishner (1991) for two *Bahua* species ( $171$  and  $35 \text{ kg s}^{-1} \text{ m}^{-1} \text{ MPa}^{-1}$ ), but higher than mean values found by Zhu & Cao (2009) in three liana species of a seasonal tropical rain forest in China ( $3.11 \text{ kg s}^{-1} \text{ m}^{-1} \text{ MPa}^{-1}$  in average). Also, Chilean species had lesser hydraulic efficiency than  $K_s$  reported for some deciduous temperate lianas from the north hemisphere, such as *Vitis riparia* and *Celastrus orbiculatus* with  $85$  and  $60 \text{ kg s}^{-1} \text{ m}^{-1} \text{ MPa}^{-1}$ , respectively (Tibbetts & Ewers 2000; Fig. 4). Although  $K_s$  values of the evergreen lianas and trees that we studied were much lower than the respective figures reported for tropical evergreen representatives of both life-forms (Ewers, Fisher & Fishner 1991; Tyree *et al.* 1991; Machado & Tyree 1994; Patiño, Tyree & Herre 1995), the  $K_s$  values we obtained from trees fell within the range of figures previously reported from other evergreen temperate forests (Brodrribb & Field 2000; Cavender-Bares & Holbrook 2001).

Conductivity of liana and tree species showed contrasting relationships with vessel size (Fig. 2a). Interspecific



**Fig. 2.** (a) Relationship between mean vessel diameter and specific hydraulic conductivity ( $K_s$ ) in (■) lianas ( $R^2 = 0.982$ ,  $P = 0.001$ ) and (□) trees ( $R^2 = 0.645$ ,  $P = 0.009$ ) and (b) relationship between mean vessel diameter and vessel density in (■) lianas ( $R^2 = 0.792$ ,  $P = 0.043$ ) and (□) trees ( $R^2 = 0.771$ ,  $P = 0.001$ ).

variation in hydraulic efficiency of lianas was positively correlated with vessel size (Fig. 2a), as has been documented in other woody plants of diverse habitats (Zimmermann 1983; Ewers 1985; Ewers & Fisher 1991; Patiño, Tyree & Herre 1995; Wagner, Ewers & Davis 1998; Gorsuch, Oberbauer & Fisher 2001). Because hydraulic conductance of a vessel is directly proportional to the fourth power of its diameter (Hagen–Poiseuille law  $k \propto d^4$ , Zimmermann 1983; Ewers & Fisher 1989), relatively modest differences in mean vessel diameter can translate to large differences in hydraulic efficiency. However,  $K_s$  of the temperate rain forest trees that we studied was negatively correlated with vessel diameter (Fig. 2a); variation in  $K_s$  of trees was thus dominated overwhelmingly by differences in the number of vessels per unit of sapwood area (Fig. 2b). This pattern is difficult to explain. As all trees that we studied are long-lived species (>150 years: Tortorelli 1956) with diffuse porous wood, vessels probably have an important role in mechanical support (Tyree & Ewers 1991;

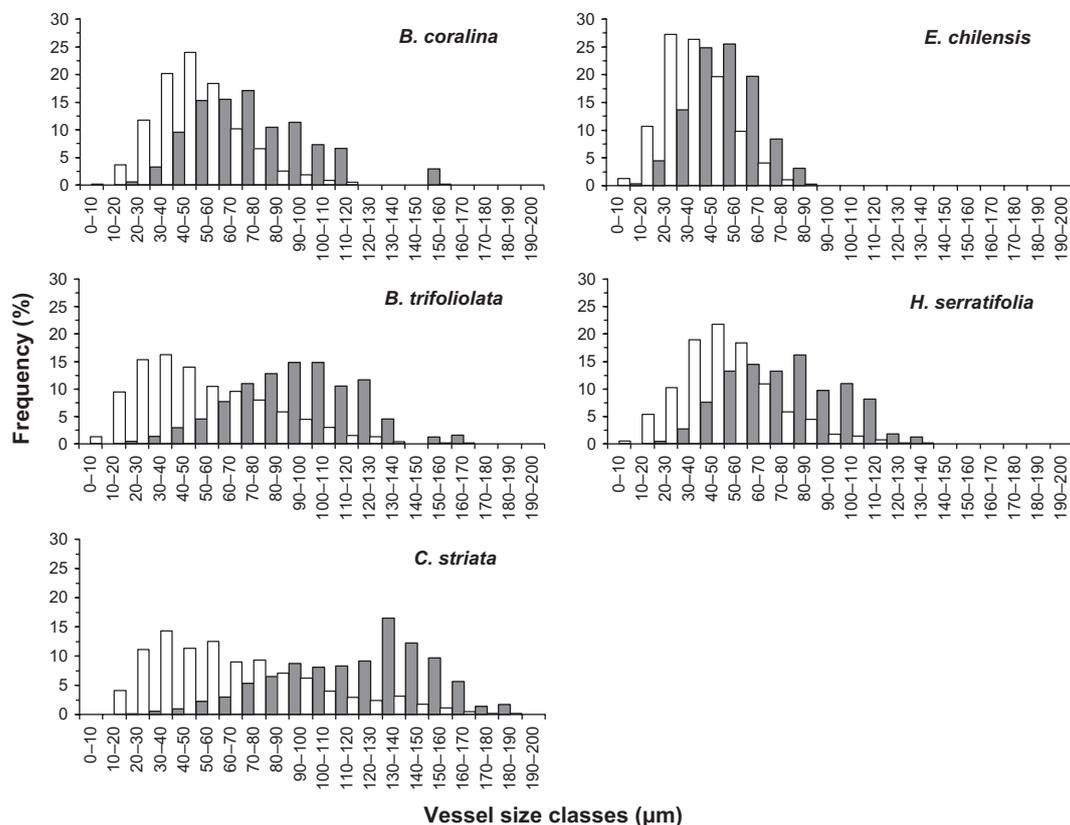


Fig. 3. Frequency distribution of xylem vessel diameters (white bars) and their relative contributions to total hydraulic conductivity (grey bars) in each liana species.

McCulloh & Sperry 2005); consequently high  $K_s$  in such plants is apparently more feasibly achieved by high vessel density than by development of wide vessel lumens, which might compromise the support function of the stem. However, this does not explain why  $K_s$  of our tree species should be negatively correlated with vessel diameters.

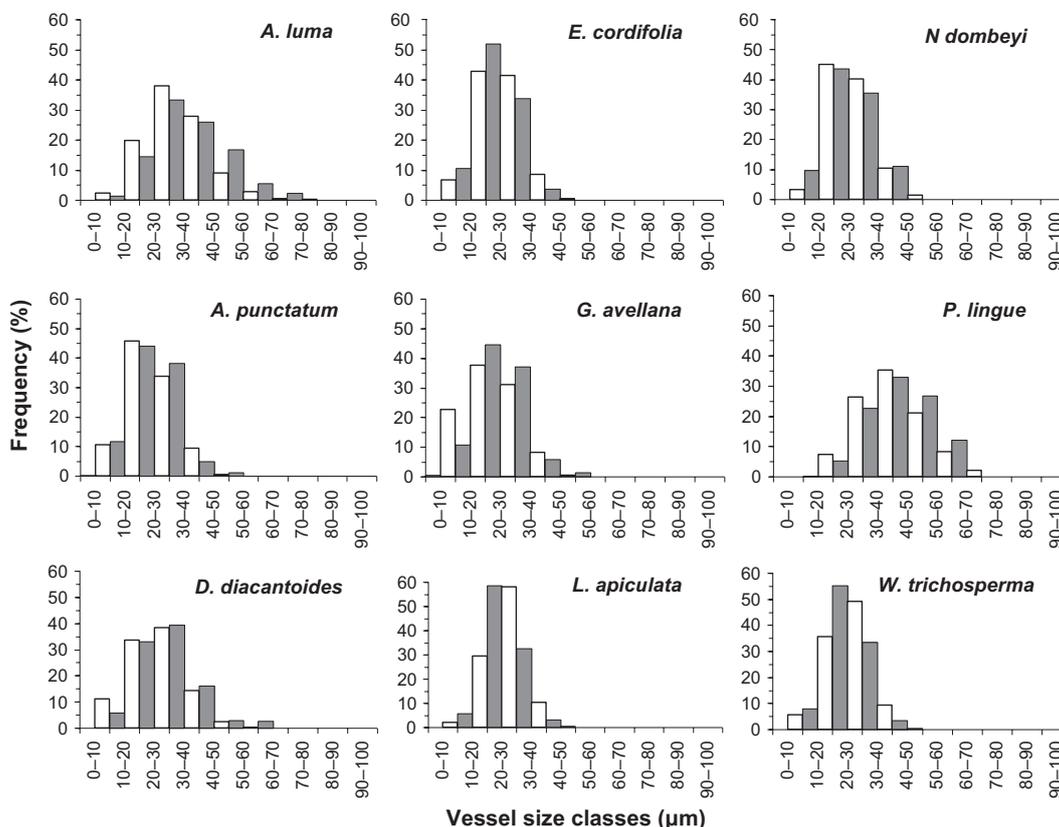
#### VULNERABILITY TO FREEZE EMBOLISM

Freeze–thaw embolism affected lianas more than trees, reducing the potential hydraulic advantage of lianas' large conduits (Table 1; Fig. 5). As far as we are aware, this is the first study to show such a pattern. Although  $K_s$  of most lianas after a frost was still considerably higher than that of the tree species, two caveats apply to this finding. First, only mild frosts occurred during the study period (Table S1), and more severe frosts might cause higher levels of embolism in lianas. Secondly, as our measurements of winter-time field  $K_s$  were taken on branches growing at heights where root pressures could be effective in counteracting embolism (Table 2), it is possible that our measurements underestimated the maximum levels of freeze–thaw embolism suffered by lianas at Nahuelbuta. This hypothesis is consistent with the data obtained from *Elytropus chilensis*, which developed the lowest root pressure of the five liana species (Table 2); the massive loss of hydraulic con-

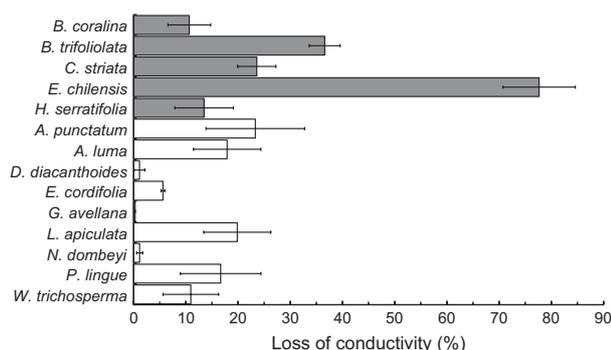
ductivity in this species might therefore be more representative than the apparently lower levels of embolism measured in the other liana species.

Across the 13 species of lianas and trees, loss of conductivity due to freeze–thaw embolism was positively correlated with vessel diameters (Fig. 6a). Although we are not aware of other studies comparing temperate rain forest lianas and trees, our findings are in agreement with several other studies, showing that conduit diameters influence susceptibility to freeze–thaw embolism (Sperry & Sullivan 1992; Davis, Sperry & Hacke 1999; Pate & Canny 1999; Field & Brodribb 2001). Deciduous species with large vessels can suffer complete loss of xylem conductivity in winter (Sperry *et al.* 1987; Tibbetts & Ewers 2000), whereas taxa with tracheids rather than vessels suffer minimal loss of conductivity even after severe frosts (Field & Brodribb 2001). There is thus substantial evidence that evolution of xylem anatomy is subject to a trade-off between high performance under frost-free conditions, and resistance to freeze–thaw embolism.

Besides the wide vessels responsible for a large fraction of water transport, liana xylem also contained many small vessels (Fig. 3), the functional role of which is not clear. Nonetheless, it is probable that a big fraction of these vessels could be functional after freezing due to milder temperatures in our study area, explaining why  $K_s$  was still very



**Fig. 4.** Frequency distribution of xylem vessels diameters (white bars) and their relative contributions to total hydraulic conductivity (grey bars) in each tree species.



**Fig. 5.** Freeze embolism measured as the percentage of loss conductivity caused for the embolism in branches of lianas (grey bars) and trees (white bars) in Cordillera Nahuelbuta (mean  $\pm$  1 SE).

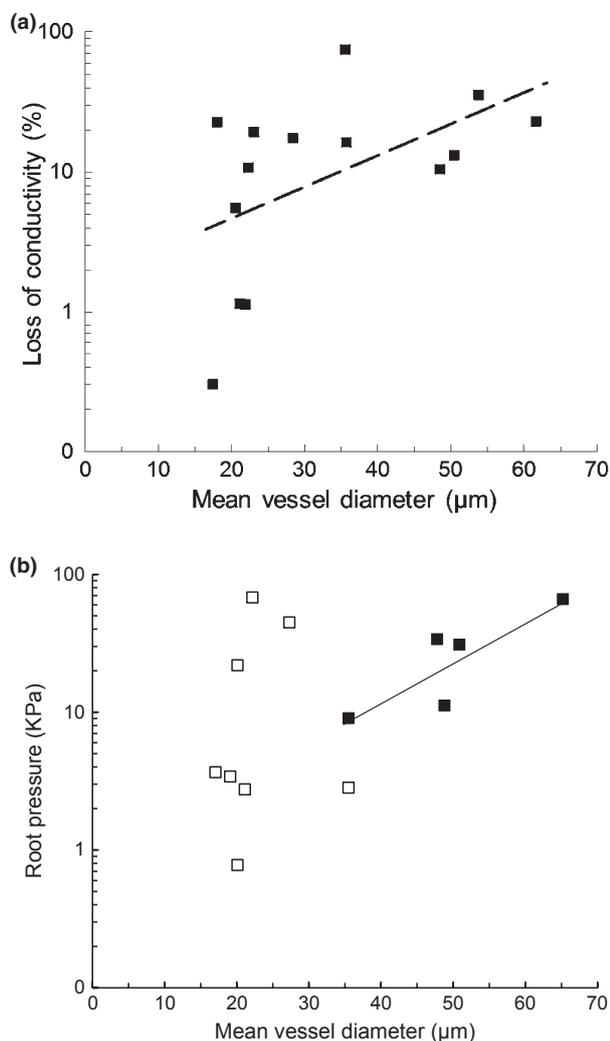
much higher in lianas than trees after freeze–thaw. Similar right-skewed distributions of vessel diameters have been reported for other tropical and temperate lianas (Ewers, Fisher & Chiu 1990; Ewers, Fisher & Fishner 1991; Chiu & Ewers 1992). While there has been little work on the significance of the numerous small vessels, it is clear that their contribution to total hydraulic conductivity is small (Fig. 3). These small vessels could act as a backup system, which supplies water to leaves when larger vessels suffer embolism (Ewers 1985), and could thus serve to expand the growing season in temperate regions (Chiu & Ewers

1992). They could also have structural functions and/or could be involved in storage or lateral movement of water (Ewers, Fisher & Fishner 1991).

#### ROOT PRESSURE

All liana species sampled in this study showed the ability to generate root pressure (Table 2). In contrast, Ewers, Cochard & Tyree (1997) found that only three of 29 vine species in a tropical forest developed root pressures. This result supports the idea that one of the main functions of root pressure is refilling embolized vessels in cold environments (Sperry *et al.* 1987; Ewers, Cochard & Tyree 1997; Tibbetts & Ewers 2000). Moreover, we found that root pressure was well correlated with vessel diameter in lianas (Fig 6b), suggesting that those species most at risk from freeze embolism invest more resources in producing high root pressure. This mechanism implies energetic costs because root pressure is generated osmotically (Isnard & Silk 2009).

The root pressures produced by lianas were not sufficient to refill embolized vessels to heights above *c.* 7 m (Table 2), indicating only a partial refilling of xylem in large lianas that reach the forest canopy. This seems to be typical of root pressures in general, although Fisher *et al.* (1997) reported that the root pressure generated by one tropical liana of the genus *Cissus* was sufficient to refill



**Fig. 6.** (a) Relationship between mean vessel diameter and percentage of loss conductivity in all lianas and trees studied ( $R^2 = 0.311$ ,  $P = 0.037$ ) and (b) relationship between mean vessel diameter and root pressure in (■) lianas ( $R^2 = 0.827$ ,  $P = 0.032$ ) and (□) trees ( $R^2 = 0.025$ ,  $P = 0.73$ ).

vessels to a height of 14.8 m. Root pressures may therefore be of greatest significance to juvenile lianas, which are in fact the individuals most likely to experience severe frosts during temperature inversions. Although some tree species also showed root pressure, this was not related to vessel diameter (Fig. 6b). As root pressure is a trait present mainly in temperate forest lianas, and as a climbing habit has evolved independently in many families and at different times along the evolution of seed plants (Gianoli 2004), we can suppose that root pressure probably has been selected as an adaptive trait in temperate lianas.

## Conclusions

The results reported here support the hypothesis that global patterns of liana distribution are a consequence of the inherent vulnerability of this life-form to freeze–thaw

**Table 2.** Root pressure measured in lianas (\*) and trees, and the maximum height that the respective root pressure can refill embolized vessels

Species	Root pressure (kPa)	Critical height for reverting embolism (m)
<i>Berberidopsis coralina</i> *	34.2	4.02
<i>Boquila trifoliolata</i> *	31.3	3.70
<i>Cissus striata</i> *	66.7	7.12
<i>Elytropus chilensis</i> *	9.2	1.72
<i>Hydrangea serratifolia</i> *	11.3	1.72
<i>Aextoxicon punctatum</i>	3.5	1.80
<i>Amomyrtus luma</i>	45.2	5.56
<i>Dasyphyllum diacanthoides</i>	2.8	1.60
<i>Eucryphia cordifolia</i>	0.8	1.47
<i>Gevuina avellana</i>	3.7	1.99
<i>Luma apiculata</i>	68.6	8.12
<i>Nothofagus dombeyi</i>	22.1	3.60
<i>Persea lingue</i>	2.9	1.10
<i>Weinmannia trichosperma</i>	0.0	0.00

embolism. As far as we are aware, this study is the first to show that, like their tropical counterparts, temperate rain forest lianas have a large potential hydraulic advantage over associated trees, but that this advantage is reduced by freeze–thaw embolism. Despite their higher percentage losses of transport capacity, partially embolized lianas still had higher conductivity than associated trees at our site, a pattern probably attributable to their wide ranges of conduit diameters. Although their large-diameter vessels were likely embolized after frosts, the many smaller conduits present in their xylem enabled some transport capacity to be conserved. Although lianas were also found to develop higher root pressures than associated trees, the pressures recorded were far from sufficient to completely repair embolism in tall lianas reaching the forest canopy. Root pressures may therefore be of most value to juvenile lianas of short stature. Temperate lianas appear to employ a high-risk strategy entailing significant levels of embolism in winter, but likely enabling rapid carbon gain during frost-free periods. Loss of winter carbon gain opportunities because of embolism may explain the low success of lianas in temperate rain forest, and the relatively low altitudinal limits of most liana species at Nahuelbuta and in other maritime temperate regions. Predicted climate-change scenarios, with milder winters, are likely to increase liana abundance in mid-latitudes and allow lianas to expand their ranges towards higher latitudes and elevations, changing the composition and structure of mid-latitude rain forests.

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## Supporting Information

Additional Supporting Information may be found in the online version of this article:

**Table S1.** Temperature records of study area during the sampling period time.