

Taylor S. Feild · Tim Brodribb

## Stem water transport and freeze-thaw xylem embolism in conifers and angiosperms in a Tasmanian treeline heath

Received: 10 May 2000 / Accepted: 3 November 2000 / Published online: 16 February 2001  
© Springer-Verlag 2001

**Abstract** The effect of freezing on stem xylem hydraulic conductivity and leaf chlorophyll *a* fluorescence was measured in 12 tree and shrub species from a treeline heath in Tasmania, Australia. Reduction in stem hydraulic conductivity after a single freeze-thaw cycle was minimal in conifers and the vessel-less angiosperm species *Tasmannia lanceolata* (Winteraceae), whereas mean loss of conductivity in vessel-forming angiosperms fell in the range 17–83%. A positive linear relationship was observed between percentage loss of hydraulic conductivity by freeze-thaw and the average conduit diameter across all 12 species. This supports the hypothesis that large-diameter vascular conduits have a greater likelihood of freeze-thaw cavitation because larger bubbles are produced, which are more likely to expand under tension. Leaf frost tolerances, as measured by a 50% loss of maximum PSII quantum yield, varied from –6 to –13°C, indicating that these species were more frost-sensitive than plants from northern hemisphere temperate forest and treeline communities. There was no evidence of a relationship between frost tolerance of leaves and the resilience of stem water transport to freezing, suggesting that low temperature survival and the resistance of stem water transport to freezing are independently evolving traits. The results of this study bear on the ecological importance of stem freezing in the southern hemisphere treeline zones.

**Keywords** Chlorophyll fluorescence · Freezing stress · Stem hydraulics · Treeline plants · Winteraceae

### Introduction

When xylem sap freezes, the insolubility of gases in ice results in the formation of air bubbles in the vascular system. After the ice melts, these bubbles can either dissolve back into the sap solution or they may expand to fill the entire volume of a vascular conduit, resulting in blockage of water transport (Hammel 1967; Sperry and Sullivan 1992; Hacke and Sauter 1996; Langan et al. 1997; Davis et al. 1999; Utsumi et al. 1999). The likelihood of bubble expansion following a freeze-thaw cycle increases as the magnitude of xylem tension present before freezing increases (Yang and Tyree 1992; Tyree et al. 1994). In addition, larger xylem conduits are more susceptible to freeze-thaw cavitation because more gas is frozen out of solution, which results in larger diameter bubbles that can more easily expand during thawing (Sperry and Sullivan 1992; Sperry et al. 1994; Davis et al. 1999).

Studies on northern hemisphere woody plants from North America and Eurasia have revealed the predominance of two divergent vascular strategies to cope with bubble production during freeze-thaw (Sperry and Sullivan 1992; Wang et al. 1992; Sperry et al. 1994; Hacke and Sauter 1996; Davis et al. 1999). The first of these is exhibited by conifers, where axial water flow occurs through narrow-diameter (generally <15 µm), single-celled tracheids. Freeze-thaw cavitation in conifer xylem is largely avoided because the bubbles formed in tracheids are very small and hence are easily redissolved during thawing unless xylem tensions are extremely high (Hammel 1967; Sucoff 1969; Robson et al. 1988; Sperry and Sullivan 1992; Sperry et al. 1994; Davis et al. 1999). Tolerance to freezing at the stem level in conifers appears to be associated with a large resistance of their foliage to cold temperatures (Sakai et al. 1981). Freeze-thaw cavitation avoidance in conifers presumably lengthens the growing season, enabling leaf retention and some gas exchange over the prolonged period of winter stress found in most temperate northern hemisphere forests (Chabot and Hicks 1982; Zimmermann 1983; Sprugel 1989).

T.S. Feild (✉)  
Department of Organismic and Evolutionary Biology,  
Harvard University, Cambridge, MA 02138, USA  
e-mail: tfeild@oeb.harvard.edu  
Tel.: +617-4954459, Fax: +617-4965854

T. Brodribb  
Department of Plant Science, University of Tasmania,  
GPO Box 252–55, Hobart 7001, Australia

The other common strategy occurs in winter deciduous angiosperm taxa possessing vascular systems dominated by large-diameter ( $>50\text{ }\mu\text{m}$ ), multi-celled vessel conduits up to 1 m in length. Vessels allow for a greater hydraulic efficiency compared to tracheids, but they can cavitate extensively during autumn freezing (Zimmermann 1983; Wang et al. 1992; Sperry et al. 1994; Hacke and Sauter 1996; Utsumi et al. 1999). Consequently, water supply to leaves cannot be maintained, and these plants shed their leaves during autumn (Chabot and Hicks 1982; Zimmermann 1983; Sperry et al. 1994). In addition, leaves of deciduous angiosperm species generally possess lower tolerance to freezing stress compared to evergreen conifers (Sakai et al. 1981). In spring, stem water transport capacity is restored, by either production of new large-diameter vessels (as in ring-porous trees; *Fraxinus*, and some *Quercus* species), or by refilling embolized vessels with root pressure (occurring in many diffuse-porous trees; *Acer*, *Betula*; Sperry et al. 1994; Hacke and Sauter 1996). The extent of winter embolism plays an important role in determining spring leaf-flushing time in many temperate deciduous trees (Wang et al. 1992; Sperry et al. 1994).

In contrast to the northern hemisphere, temperate forests of the southern hemisphere are dominated by woody evergreen angiosperms, and contain few species of winter-deciduous trees (e.g., one species in Australia, approximately ten species in South America, and five species in New Zealand; Axelrod 1966; Wardle 1971; Chabot and Hicks 1982; Röhrig 1991; Markgraf et al. 1995). Also, conifers play a relatively minor role in the vegetation structure when compared to the abundance of Pinaceae in the northern hemisphere boreal zone and treeline communities (Wardle 1971, 1985; Sprugel 1989). One explanation for this large difference in vegetation structure is the maritime climate on southern hemisphere land masses, producing greatly reduced seasonal variation in temperature relative to the continental climate of the northern hemisphere (Axelrod 1966; Wardle 1971, 1985; Sakai et al. 1981; Sprugel 1989; Markgraf et al. 1995). The attenuation of winter minimum and summer maximum temperatures appears to have resulted in evolution of a lower frost tolerance of southern hemisphere plants, and thus southern hemisphere treelines are lowered, on average, 1,000 m below those in the northern hemisphere at equivalent latitudes (Daubenmire 1954; Sakai et al. 1981; Wardle 1985; Sprugel 1989; Markgraf et al. 1995).

However, some southern hemisphere plant communities are frequently exposed to subfreezing temperatures (although generally not lower than  $-20^{\circ}\text{C}$ ; Markgraf et al. 1995), and until now no data have been presented to indicate what role freeze-thaw conditions play in reducing stem xylem hydraulic conductivity and the possible regulation of leaf phenology. In this study, we examined stem xylem vulnerability to freeze-thaw treatment in a co-occurring group of conifers and angiosperms from a treeline mixed conifer-heath community in Tasmania (Australia), and tested for a relationship between loss of

xylem hydraulic conductivity and xylem structure. Also, the single deciduous species in Australia, *Nothofagus gunnii*, was included to investigate whether its responses to stem xylem freezing were convergent with northern deciduous diffuse-porous angiosperms. Finally, this study investigates the relationship between freezing tolerance at the leaf and xylem level, to examine if xylem dysfunction by freezing is a causal factor in the expression of leaf freezing tolerance.

## Materials and methods

### Field site, species examined, and temperature measurements

The study was carried out at Mt. Field, Tasmania ( $42^{\circ}41'\text{S}$ ,  $146^{\circ}35'\text{E}$ ), Australia in a dwarf mixed-conifer forest, growing at the treeline (1,200 m) on a humic soil (Crowden 1999). Canopy height of the heath community was typically under 1 m, with scattered emergent conifers (*Athrotaxis*, *Diselma*, and *Microstrobos*) and *Eucalyptus* reaching 3 m tall (see Crowden 1999). Measurements of stem hydraulics were conducted from May to July 1999, and leaf frost tolerance was determined in summer (February to March 2000, see below). Rainfall at the field site is approximately 2,200 mm a year, with no appreciable dry season (Crowden 1999). Mean monthly temperatures range from a maximum of  $9.6^{\circ}\text{C}$  in February to a minimum of  $0^{\circ}\text{C}$  in July. However, day/night variations in air temperature can be large (up to  $30^{\circ}\text{C}$ ), with freezing temperatures occurring at any time of the year (Crowden 1999). Twelve woody tree and shrub species were selected for this study: three conifers [*Diselma archeri* Hook.f. (Cupressaceae), *Podocarpus lawrencei* Hook.f. (Podocarpaceae), and *Phyllocladus aspleniifolius* (Labill.) Rich ex Hook.f. (Podocarpaceae)] and nine angiosperms [*Atherosperma moschatum* Labill. (Monimiaceae), *Eucalyptus coccifera* Hook.f. (Myrtaceae), *Leptospermum rupestre* Hook.f. (Myrtaceae), *Nothofagus cunninghamii* (Hook.) Oersted (Fagaceae), *Nothofagus gunnii* (Hook.f.) Oersted (Fagaceae), *Orites revoluta* R.Br. (Proteaceae), *Ozothamnus rodwayi* Orch. (Asteraceae), *Richea scoparia* Hook.f. (Epacridaceae), and the vessel-less *Tasmannia lanceolata* (Poir.) A.C.Smith (Winteraceae)]. Two of these species, *A. moschatum* and *P. aspleniifolius*, are temperate rainforest taxa that occur as scattered individuals at a lower elevation (1,000 m, Read 1999). Freezing occurs frequently at this elevation on Mt. Field, but the extremes of minimum air temperatures during winter may be lower than at 1200 m (Crowden 1999).

Diurnal air and soil temperatures were measured with four thermistor temperature probes interfaced to a datalogger (HOBO, HTEA-27+46, Onset Computer, Mass.). For soil temperature, measurements were averages based on readings from two thermistor probes buried under 3 cm of soil. Diurnal air temperatures were measured in the sun and in the shade by suspending one of the temperature probes from a fully exposed branch of a canopy *E. coccifera* tree and placing another probe in a shaded area. Both probes were 30 cm above the soil surface. Temperature data were taken every 15 min over a 10-month period from June 1999 to April 2000.

### Stem xylem hydraulic conductivity

Stem xylem hydraulic conductivity ( $K_H$ ) was defined as the mass flow rate of water through an excised stem segment per unit pressure gradient ( $\text{kg m s}^{-1} \text{MPa}^{-1}$ ; Sperry et al. 1988). Stem segments were cut from branches at length longer than the maximum vessel length, which was determined using low pressure (0.01 MPa) air-injection (Zimmermann and Jeje 1981). Flow rates were measured with a portable low pressure flow meter, which uses the drop in pressure across a tube of known conductance to measure the flow rate into the stem segment, together with the pressure at

the stem fitting, to determine stem  $K_H$  (Feild and Holbrook 2000; Zwieniecki et al., 2000). The flow meter was constructed and operated as described in Feild and Holbrook (2000). Stems were measured under delivery pressures ranging from 0.003 to 0.005 MPa and data were recorded when steady-state pressure readings were achieved (typically 10 min) after connection to flow meter. During all measurements, stem temperature was measured with a copper-constantan thermocouple (30 American wire gauge, Omega Engineering, Stamford, Conn.) so that the effect of temperature on viscosity of water flux could be removed by normalizing all measurements to 20°C (Sperry et al. 1988).

Sapwood-specific conductivity ( $K_{Sp}$ ;  $\text{kg m}^{-1} \text{s}^{-1} \text{MPa}^{-1}$ ) was determined by dividing maximum stem  $K_H$  by the sapwood cross-section area. Maximum stem  $K_H$  was determined after subjecting stems to a series (two to three) of high-pressure flushes (175 kPa) with distilled, filtered (to 0.2  $\mu\text{m}$ ) water to remove emboli (Sperry et al. 1988). During the study period, measurements of stem  $K_H$  before and after flushing revealed that "native embolism" for all vessel-forming species was less than 8% (data not shown). Considering that high-pressure flushing was an ineffective means of determining maximum stem  $K_H$  in conifers (Sperry and Tyree 1990; Brodribb and Hill 1999) and the vessel-less angiosperm *T. lanceolata*, (T. Feild, unpublished data), the initial stem  $K_H$  was assumed to be non-embolized, and therefore branches for hydraulic and freeze-thaw treatments were sampled between 0900 hours and 1100 hours on rainy, cloudy days when embolism was assumed to be low. Sapwood cross-section was measured with a micrometer along the major and minor axes of the distal stem end (with the bark removed), and the surface area contributed by the pith was subtracted (Sperry et al. 1988).

#### Freeze-thaw treatment and changes in stem hydraulic conductivity

Before freeze-thaw treatment, leaf-bearing branches were dehydrated to a moderate stem xylem tension ( $-0.5 \text{ MPa}$ ) to approximate conditions likely experienced in the field when plants undergo a freeze-thaw cycle (T. Brodribb, unpublished data). Drought-stress vulnerability curves for *Podocarpus lawrencei* and *T. lanceolata* indicated that no embolism should be induced by dehydration to a  $\Psi_{\text{stem}}$  of  $-0.5 \text{ MPa}$  (Brodribb and Hill 1999; T. Feild, unpublished data).  $\Psi_{\text{stem}}$  was inferred by measuring the leaf water potential with a Scholander pressure bomb, after a dehydrated branch was placed into a humid plastic bag in the dark for 30 min (Sperry et al. 1988). Branches were frozen in humid plastic bags to  $-10^\circ\text{C}$  at a controlled freezing rate of  $1^\circ\text{C}/\text{min}$  in a frost cabinet as described by Read and Hill (1988). Freezing exotherms, which occurred between  $-2$  and  $-6^\circ\text{C}$  in all species, were measured on portions of the branches not used for hydraulic measurements by inserting a copper-constantan thermocouple (30 American gauge wire size, Omega Engineering, Stamford, Conn.) between the bark and wood. After 2 h, bagged branches were thawed to room temperature ( $16$ – $18^\circ\text{C}$ ), which required approximately 150 min. The temperature for freezing branches was selected to ensure that samples underwent freezing, but avoiding freezing-induced damage to living xylem cells that can reduce stem  $K_H$  in ways independent of xylem embolism (Pockman and Sperry 1997; T. Feild, unpublished data). Measurements of the relationship between the loss of stem  $K_H$  (relative to an initial value before treatment, see below) and freezing temperature for a subsample of the 12 species investigated (e.g., *Eucalyptus*, *N. cunninghamii*, *Orites*, *Phyllocladus*, *Podocarpus*, *Tasmannia*) suggest that damage to living vascular tissues did not occur until freezing temperature dropped below  $-15^\circ\text{C}$  (T. Feild, unpublished data). Thus, at a freezing temperature of  $-10^\circ\text{C}$ , we assume that decreases in stem  $K_H$  following freeze-thaw result from xylem embolism.

The loss of stem  $K_H$  following freeze-thaw treatment was determined using two methods. In the first method, stem  $K_H$  was measured after freezing and then compared with a maximum stem  $K_H$  produced after the stem was treated with three 20-min 175 kPa flushes of distilled, filtered water to calculate percentage loss in stem  $K_H$  (PLC, Sperry et al. 1988). The paired-segment method was used for measuring the response of vessel-less xylem to freez-

ing (Sperry and Sullivan 1992, Sperry et al. 1994). When branches reached the desired  $\Psi_{\text{stem}}$ , two segments (approximately 10 cm in length each, with 1 cm between them) were marked on a leaf-bearing branch with no significant taper. One stem segment was excised in air, trimmed underwater to remove embolized tracheids, and then measured while the remaining portion of the branch was frozen under tension. After freeze-thaw treatment, stem  $K_H$  was measured and compared to the stem  $K_H$  of the first stem segment. The percentage that stem  $K_H$  of the second stem segment was below that of the first segment  $K_H$  gave the percentage loss in stem  $K_H$  resulting from freeze-thaw (Sperry and Sullivan 1992; Sperry et al. 1994).

#### Xylem conduit diameter

Conduit diameters were measured on transverse sections from three stem segments of each species that were measured for stem  $K_H$ . The major and minor axes of all tracheids and vessels present in a given sector of the most recent growth ring were measured with a light microscope micrometer at  $\times 1,000$ . Sectors were located randomly around the stem cross-section, excluding regions of compression wood in conifers and tension wood in angiosperms, which conduct little water (Spicer and Gartner 1998). For each sector, 100 conduits were measured, and a mean diameter was determined for each species ( $n=3$  stems for each species).

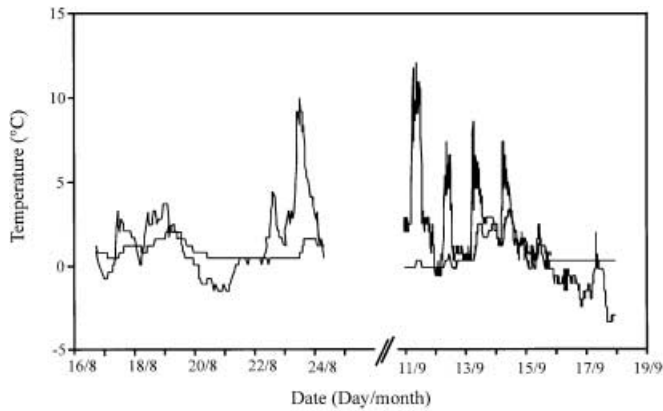
#### Leaf sensitivity to freezing

The viability of leaves following treatment at several subfreezing treatments was determined with chlorophyll fluorescence measurements (Neuner and Bannister 1995; Boorse et al. 1998; Neuner and Buchner 1999) using a pulse-amplitude modulated fluorometer (PAM-2000, Walz, Effeltrich, Germany). Twenty branches from each species were collected at Mt. Field and placed in humid, dark bags for 7 h at  $20^\circ\text{C}$  to ensure maximum re-oxidation of photosystem II (PSII) reaction centers (Schreiber et al. 1994). Maximum PSII quantum yield (ratio of variable to maximal fluorescence yield,  $F_v/F_m$ , Schreiber et al. 1994) was determined during the application of a  $3500 \mu\text{mol photons m}^{-2} \text{s}^{-1}$  flash for 800 ms on the dark-adapted leaves. Following measurement of a control value of  $F_v/F_m$ , four branches of each species were frozen for 1 h at temperatures of  $-5$ ,  $-7.5$ ,  $-10$ ,  $-15$ , and  $-20^\circ\text{C}$ . After a thawing period of 24 h in darkness at  $20^\circ\text{C}$ ,  $F_v/F_m$  was re-measured on three leaves from each of the four branches at each treatment temperature. The period of dark-adaptation was kept long to allow for potential recovery through repair and synthesis of photosynthetic membranes and PSII following freezing treatment (Schreiber et al. 1994). In each species the relationship between loss of fluorescence and freezing temperature was best approximated by a cumulative normal distribution curve, so data were probit-transformed and regressions fitted. From these regressions, the temperature corresponding to a 50% loss of  $F_v/F_m$  was interpolated. Measurements were conducted during a 1-week period in summer (February 2000) so that results for the winter-deciduous plant *N. gunnii* could be compared to the rest of the evergreen species included in this study. Limited observations on six species of evergreen conifers and angiosperms (the same taxa used as above) during winter demonstrated that some winter hardening ( $3$ – $5^\circ\text{C}$  lowering of the 50% loss of  $F_v/F_m$  compared to summer values) can occur (see also Read and Hill 1988). However, the ordering of these six species' tolerance during winter did not differ from that observed in summer (T. Feild, unpublished data).

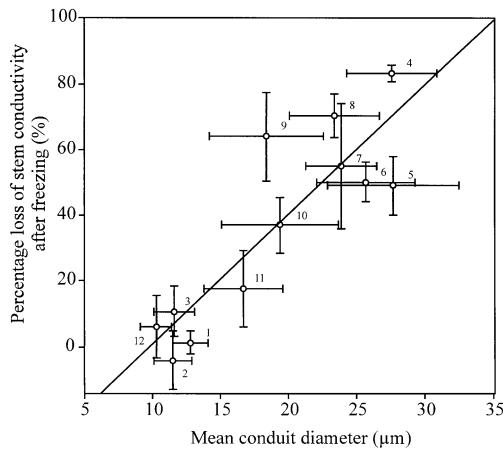
## Results

During the winter months of 1999, air temperatures ranged from  $-4^\circ\text{C}$  to  $12.5^\circ\text{C}$  and soil temperatures ranged from  $-2^\circ\text{C}$  to  $4^\circ\text{C}$  (Fig. 1). A representative peri-





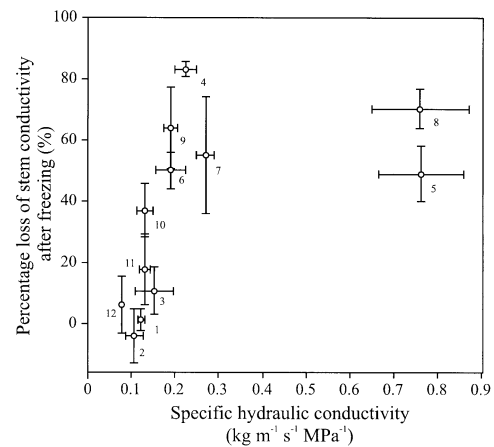
**Fig. 1** Representative diurnal variation in winter air and soil temperatures in a treeline mixed-conifer heath, Tasmania (Australia). Soil temperature ( $^{\circ}\text{C}$ ) is indicated by the bold line and air temperature is denoted by the gray line



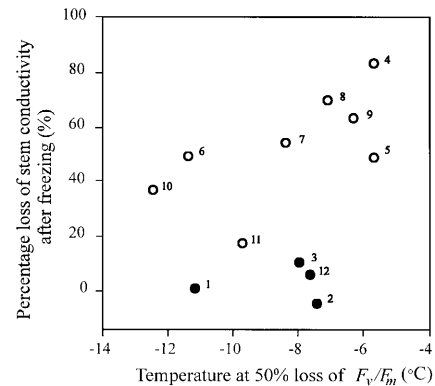
**Fig. 2** The relationship between average ( $n=4$ ) percentage loss in stem hydraulic conductivity after a single freeze-thaw cycle and the average ( $n=3$  stems) xylem conduit diameter. Error bars are SE. Species are labeled: 1 *Podocarpus lawrencei*; 2 *Diselma archeri*; 3 *Phyllocladus aspleniifolius*; 4 *Atherosperma moschatum*; 5 *Eucalyptus coccifera*; 6 *Nothofagus cunninghamii*; 7 *Leptospermum rupestre*; 8 *Nothofagus gunnii*; 9 *Orites revoluta*; 10 *Ozothamnus rodwayi*; 11 *Richea scoparia*; 12 *Tasmania lanceolata*

od of diurnal air and soil temperature is depicted in Fig. 1. Typically, relatively cold days were followed by warming periods lasting several days during mid-winter, which gradually increased the soil temperatures from zero to as much as  $3^{\circ}\text{C}$ .

In conifers and the vessel-less angiosperm *T. lanceolata*, mean percentage losses of hydraulic conductivity (PLC) after a single freeze-thaw cycle were not significantly different from zero. In contrast, mean PLC in associated evergreen vessel-forming angiosperm species were found to range from 18% in *Richea scoparia* to 83% in *Atherosperma moschatum* (Fig. 2). Mean PLC in the single deciduous species measured, *N. gunnii*, was approximately  $70.3 \pm 6.5\%$  (Fig. 2). Average conduit di-



**Fig. 3** Average ( $n=4$ ) percentage loss of hydraulic conductivity by freezing in relation to average ( $n=4$ ) sapwood specific hydraulic conductivity ( $K_{\text{sp}}$ ,  $\text{kg m}^{-1} \text{MPa}^{-1} \text{s}^{-1}$ ) for each of the 12 species (labeled as in Fig. 1)



**Fig. 4** The relationship between average ( $n=4$ ) percentage loss in stem hydraulic conductivity by freeze-thaw treatment and the temperature resulting in 50% loss of the variable to maximal chlorophyll fluorescence ( $F_v/F_m$ ) yield for each of the 12 species (labeled as in Fig. 1). Vessel-less taxa are denoted by filled circles and unfilled circles represent vessel-bearing angiosperms

ameter varied from 10 mm in the vessel-free xylem of *T. lanceolata* to 28  $\mu\text{m}$  in *E. coccifera* (Fig. 2). A strong linear relationship ( $r^2=0.758$ ;  $P<0.01$ ) was found to exist between mean conduit diameter and PLC after a single freeze-thaw (Fig. 2). This did not correspond to a good correlation between  $K_{\text{sp}}$  and PLC, although species with higher  $K_{\text{sp}}$  tended to be more susceptible to freeze-thaw embolism (Fig. 3).

Leaf frost tolerances varied among species (Fig. 4). The most vulnerable leaves were produced by *A. moschatum* and *E. coccifera*, with both exhibiting a 50% loss in maximum PSII quantum yield ( $F_v/F_m$ ) at an average temperature of  $-5.6^{\circ}\text{C}$  whereas, at the other extreme, leaves of *Ozothamnus rodwayi* on average had lost 50%  $F_v/F_m$  at a temperature of  $-12.5^{\circ}\text{C}$ . No significant difference in freezing tolerance of leaves from vessel-less and vessel-bearing taxa was observed. No relationship was found to exist between xylem vulnerability to freeze-

thaw cavitation and the freezing tolerance of leaves (Fig. 4), although species with the highest PLCs after freezing tended to be most susceptible to frost damage in their leaves, especially if the vessel-less taxa were considered as a separate group.

## Discussion

We found considerable variation in stem xylem vulnerability to freeze-thaw treatment among the 12 species of co-occurring conifers and angiosperms from a treeline heath community in Tasmania. All evergreen vessel-forming angiosperms exhibited some loss of stem  $K_H$  due to stem freezing and thawing, with percentage loss of stem conductivity (PLC) increasing sharply with average conduit diameter (Fig. 2). Specifically, PLC increased from mean values around zero in conifers and a vessel-less angiosperm with average conduit diameter below 15  $\mu\text{m}$  diameter, to more than 70–80% in plants with average conduit diameter near 30  $\mu\text{m}$  (Fig. 2). The freeze-thaw hydraulic responses of the southern conifers (*Diselma*, *Phyllocladus*, and *Podocarpus*) were similar to those measured in other studies on northern conifer genera (e.g., *Abies*, *Larix*, *Picea*; Wang et al. 1992; Sperry et al. 1994; Davis et al. 1999). In addition, the winter-deciduous tree, *N. gunnii*, had a convergent freeze-thaw hydraulic response to many northern hemisphere diffuse-porous species (e.g., some species of *Acer* and *Betula*; Sperry et al. 1994), losing approximately 70% of stem hydraulic conductivity following a single freeze-thaw event. The relationship shown here between loss of hydraulic conductivity and conduit size among all species appears consistent with the hypothesis that larger diameter vascular conduits have greater probability of freeze-thaw cavitation because larger bubbles present during thawing expand more easily under tension (Yang and Tyree 1992; LoGullo and Salleo 1993; Sperry et al. 1994; Tyree et al. 1994; Pockman and Sperry 1997; Davis et al. 1999).

The continuum in mean xylem conduit dimension and loss of stem  $K_H$  found here (Fig. 2) contrasts with the relationships observed in many northern hemisphere species where two divergent ecological groups are predominant. These groups are the winter-deciduous element with large conduits and an extremely high susceptibility to freeze-thaw embolism, and winter evergreens, which tend to produce small conduits resistant to freeze embolism (Sperry and Sullivan 1992; Wang et al. 1992; Sperry et al. 1994; Hacke and Sauter 1996; Davis et al. 1999). Although species with resistant xylem were found in our sample of plants, there was no equivalent to the extreme conduit size/embolism sensitivity exhibited by the ring-porous deciduous element of the northern hemisphere (Sperry et al. 1994; Davis et al. 1999). This is presumably due to the relatively small range of temperature from summer to winter in the southern hemisphere, which may make the ring-porous deciduous strategy non-competitive (Markgraf et al. 1995).

Another interesting contrast with data from northern hemisphere species is that we found a different relationship between conduit size and loss of stem  $K_H$  following freeze-thaw compared to that shown in studies using North American species (Sperry and Sullivan 1992; Sperry et al. 1994; Davis et al. 1999). These studies concluded that species with mean conduit diameters <30  $\mu\text{m}$  were resistant to embolism while species with a mean conduit diameter of >40  $\mu\text{m}$  were nearly completely cavitated by a single freeze-thaw treatment. In contrast, we found all species with mean conduit diameter >15  $\mu\text{m}$  were sensitive to freeze-thaw embolism (Fig. 2). The greater resistance reported for northern hemisphere species may reflect the use of slower thawing rates (generally 0.45°C/h) in these studies, as compared to the faster thawing rate (8.4°C/h) used here (Langan et al. 1997). Slower rates of thawing may decrease the likelihood of xylem embolism because more time is available for bubbles to re-dissolve in the xylem sap (Langan et al. 1997). However, the faster thawing rates used in this study are close to those observed in habitats analogous to the summer conditions of alpine Tasmania (i.e., tropical montane alpine zones of Hawaii; Melcher et al. 2000). Thawing rates of stems in winter are also potentially rapid when northerly wind patterns become occasionally dominant, resulting in air temperatures that can range from –4°C at predawn to as high as 15°C at midday (Crowden 1999). Another potential difference is at the level of wood structure. We found that the anatomy of all of the taxa we investigated possessed large geometric deviations from ideal cylindrical tubes, resulting from their angular conduit cross-section, frequent scalariform perforation plates, and/or oblique conduit taper, which could reduce the strength of the correlation between conduit diameter and occurrence of freeze-thaw embolism. Some support for this suggestion is provided by a report of a northern hemisphere evergreen shrub (e.g., *Rhododendron*), which possesses angular vessels and exhibited freeze-thaw embolism despite having a mean vessel diameter less than 30  $\mu\text{m}$  (Lipp and Nilsen 1997). Clearly, more work is needed at the anatomical level to link the changes in hydraulic responses to freeze-thaw at the stem level to vascular conduit geometry.

The dominance of evergreen taxa at the treeline that are freeze-thaw sensitive suggests that opportunistic growth during winter is favoured in the current climate of Tasmania. Winter-deciduous taxa such as *N. gunnii* with prolonged dormancy are unable to capitalize on growth conditions over winter, and are therefore restricted to the coldest limits of forest growth in Tasmania. Consistent with this interpretation, the abundance of *N. gunnii* pollen from cores at Darwin Crater, Tasmania, increased during colder glacial climates and declined with the onset of warmer interglacial periods over the last 2 million years (Colhoun 2000).

We did not find any evidence for a linkage between the extent of freeze-thaw-induced loss of stem hydraulic conductivity and leaf freezing resistance among the species investigated (Fig. 4). This suggests that low temper-

ature survival and the resistance of wood to freeze-thaw embolism are not necessarily co-evolving traits. However, additional studies should be made to determine whether the effects of freezing stress on the living components of wood (rays and xylem parenchyma) and aspects of xylem transport that are dependent on metabolic activity (e.g., embolism repair; Holbrook and Zwieniecki 1999) are similar to those of leaf tissue. In addition, these studies could be conducted in a robust phylogenetic framework to examine the comparative responses of closely related taxa that have radiated in freezing-prone versus non-freezing climates. Freezing tolerances of evergreen angiosperms measured in this study with chlorophyll *a* fluorescence were similar to those found in previous studies of evergreen trees species from comparable latitudes and altitudes in Tasmania, Chile, and New Zealand (Sakai and Wardle 1978; Sakai et al. 1981; Alberti et al. 1985; Read and Hill 1988; Reitsma 1994). The leaves of southern conifers were substantially less tolerant of freezing (with temperatures of 50% loss in maximum PSII quantum yield ranging from  $-7.5^{\circ}\text{C}$  in *Phyllocladus aspleniifolius* to  $-11.5^{\circ}\text{C}$  in *Diselma archeri*) than all conifers previously measured in northern hemisphere boreal and temperate forest zones, which can tolerate temperatures as low as  $-40^{\circ}\text{C}$  (Sakai et al. 1981).

The large range of freeze-thaw xylem cavitation responses found among co-occurring evergreen woody plants in the Tasmanian treeline adds a new dimension to the ecological importance of stem freezing. In contrast to northern hemisphere forests, seasonal growth in many evergreen angiosperm trees and shrubs at the treeline appears to be opportunistic, as indicated by the production of freeze-thaw cavitation-prone xylem, despite the occurrence of frequent freezing events. Cavitation in evergreen treeline plants in Tasmania can apparently be tolerated because many of the species measured here grow during winter (Crowden 1999). One possible explanation for the success of evergreen taxa at the treeline in spite of frequent freeze-thaw cycles is that cavitated conduits may be refilled, possibly by root pressure. Consistent with this suggestion is the fact that winter soil temperatures rarely fall below zero and sometimes reach as high as  $5^{\circ}\text{C}$ , which may permit root pressure activity in angiosperms (Fig. 1). In taxa with limited ability for root pressure, such as conifers and possibly Winteraceae, avoidance of freeze-thaw cavitation is provided by reliance on small-diameter tracheids for water transport, which represents another solution to the frequent occurrence of freeze-thaw sequences. Future studies will be required to test these predictions.

**Acknowledgements** We would like to thank R.S. Hill, N.M. Holbrook, G. Jordan, and P.J. Melcher for discussions and comments on the manuscript. This research was funded by an NSF dissertation enhancement grant to T.S. Feild (IBN-9902239) and a large ARC grant to T. Brodribb.

## References

- Alberti M, Romero M, Rios D, Wenzel H (1985) A latitudinal gradient of frost resistance in *Nothofagus* communities. *Oecol Plant* 6:21–30
- Axelrod DI (1966) Origin of deciduous and evergreen habits in temperate forests. *Evolution* 20:1–15
- Boorse GC, Gartman TL, Meyer AC, Ewers FW, Davis SD (1998) Comparative methods of estimating freezing temperatures and freezing injury in leaves of chaparral shrubs. *Int J Plant Sci* 159:513–521
- Brodribb T, Hill RS (1999) The importance of xylem constraints in the distribution of conifer species. *New Phytol* 143:365–372
- Chabot BF, Hicks DJ (1982) The ecology of leaf life spans. *Annu Rev Ecol Syst* 13:229–259
- Colhoun EA (2000) Vegetation and climate change during the last interglacial-glacial cycle in western Tasmania, Australia. *Palaeogeogr Palaeoclimatol Palaeoecol* 155:195–209
- Crowden RK (1999) Alpine vegetation. In: Reid JB, Hill RS, Brown MJ, Hovenden MJ (eds) *Vegetation of Tasmania*. ABRIS, Canberra, pp 333–356
- Daubenmire R (1954) Alpine timberlines in the Americas and their interpretation. *Butler Univ Bot Stud* 11:119–136
- Davis SD, Sperry JS, Hacke UG (1999) The relationship between xylem conduit diameter caused by freeze-thaw events. *Am J Bot* 86:1341–1355
- Feild TS, Holbrook NM (2000) Xylem sap flow and stem hydraulics of the vessel-less angiosperm *Drimys granadensis* (Winteraceae) in a Costa Rican elfin forest. *Plant Cell Environ* 23:1067–1077
- Hacke U, Sauter JJ (1996) Xylem dysfunction during winter and recovery of hydraulic conductivity in diffuse-porous and ring-porous trees. *Oecologia* 105:435–439
- Hammel HT (1967) Freezing of xylem sap without cavitation. *Plant Physiol* 42:55–66
- Holbrook NM, Zwieniecki MA (1999) Embolism refilling and xylem tension: do we need a miracle? *Plant Physiol* 120:7–10
- Langan SJ, Ewers FW, Davis SD (1997) Xylem dysfunction caused by water stress and freezing in two species of co-occurring chaparral shrubs. *Plant Cell Environ* 20:425–437
- Lipp CC, Nilsen ET (1997) The impact of subcanopy light environment on the hydraulic vulnerability of *Rhododendron maximum* to freeze-thaw cycles and drought. *Plant Cell Environ* 20:1264–1272
- LoGullo MA, Salleo S (1993) Different vulnerabilities of *Quercus ilex* L. to freeze- and summer drought-induced xylem embolism: an ecological interpretation. *Plant Cell Environ* 16:511–519
- Markgraf V, McGlone M, Hope G (1995) Neogene palaeoenvironmental and paleoclimatic change in southern temperate ecosystems – a southern perspective. *Trend Ecol Evol* 10:143–147
- Melcher PJ, Cordell S, Jones T, Giambelluca T, Scowcroft P, Goldstein G (2000) Supercooling capacity increases from sea level to treeline in the Hawaiian tree species *Metrosideros polymorpha*. *Int J Plant Sci* 16:369–379
- Neuner G, Bannister P (1995) Frost resistance and susceptibility to ice formation during natural hardening in relation to leaf anatomy in three evergreen tree species. *Tree Physiol* 15:371–377
- Neuner G, Buchner O (1999) Assessment of foliar frost damage: a comparison of in vivo chlorophyll fluorescence with other viability methods. *J Appl Bot* 73:50–54
- Pockman WT, Sperry JS (1997) Freezing-induced xylem cavitation and the northern limit of *Larrea tridentata*. *Oecologia* 109:19–27
- Read J (1999) Rainforest ecology. In: Reid JB, Hill RS, Brown MJ, Hovenden MJ (eds) *Vegetation of Tasmania*. ABRIS, Canberra, pp 333–356
- Read J, Hill RS (1988) The comparative responses to temperature of some Tasmanian rainforest tree species. I. Foliar frost resistance. *Aust J Bot* 36:131–143
- Reitsma L (1994) The frost resistance of some native plants from the central volcanic plateau, North Island, New Zealand in relation to forest succession. *NZ J Bot* 32:217–226

- Robson DJ, McHardy WJ, Petty JA (1988) Freezing in conifer xylem. II. Pit aspiration and bubble formation. *J Exp Bot* 39: 1617–1621
- Röhrig E (1991) Seasonality. In: Röhrig E, Ulrich B (eds) *Ecosystems of the world*, vol 7. Temperate deciduous forests. Elsevier, New York, pp 25–33
- Sakai A, Wardle P (1978) Freezing resistance of New Zealand trees and shrubs. *NZ J Ecol* 1:51–61
- Sakai A, Paton DM, Wardle P (1981) Freezing resistance of trees of the south temperate zone, especially sub-alpine species of Australasia. *Ecology* 62:563–570
- Schreiber U, Bilger W, Neubauer C (1994) Chlorophyll fluorescence as a non-intrusive indicator for rapid assessment of in vivo photosynthesis. In: Schulze ED, Caldwell MM (eds) *Ecophysiology of photosynthesis*. Springer, Berlin Heidelberg New York, pp 49–70
- Sperry JS, Sullivan JEM (1992) Xylem embolism in response to freeze-thaw cycles and water stress in ring-porous, diffuse-porous, and conifer species. *Plant Physiol* 100:605–613
- Sperry JS, Tyree MT (1990) Water-stress-induced xylem embolism in three species of conifers. *Plant Cell Environ* 19:427–436
- Sperry JS, Donnelly JR, Tyree MT (1988) A method for measuring hydraulic conductivity and embolism in xylem. *Plant Cell Environ* 11:35–40
- Sperry JS, Nicols KL, Sullivan JEM, Eastlack SE (1994) Xylem embolism in ring-porous, diffuse-porous, and coniferous trees of northern Utah and interior Alaska. *Ecology* 75:1736–1752
- Spicer R, Gartner BL (1998) Hydraulic properties of Douglas-fir (*Pseudotsuga menziesii*) branches and branch halves with reference to compression wood. *Tree Physiol* 18:777–784
- Sprugel DG (1989) The relationship of evergreenness, crown architecture, and leaf size. *Am Nat* 133:465–479
- Suocoff E (1969) Freezing in conifer xylem sap and the cohesion-tension theory. *Physiol Plant* 22:424–431
- Tyree MT, Davis SD, Cochard H (1994) Biophysical perspectives of xylem evolution: is there a tradeoff of hydraulic efficiency for vulnerability to dysfunction? *IAWA J* 15:335–360
- Utsumi Y, Sano Y, Funada R, Fujikawa S, Ohtani J (1999) The progression of cavitation in earlywood vessels of *Fraxinus mandshurica* var *japonica* during freezing and thawing. *Plant Physiol* 121:897–904
- Wang J, Ives NE, Lechowicz MJ (1992) The relation of foliar phenology to xylem embolism in trees. *Funct Ecol* 6:469–475
- Wardle P (1971) An explanation for alpine timberline. *NZ J Bot* 9:371–402
- Wardle P (1985) New Zealand timberlines. 3. A synthesis. *NZ J Bot* 23:263–271
- Yang S, Tyree MT (1992) A theoretical model of hydraulic conductivity recovery from embolism with comparison to experimental data on *Acer saccharum*. *Plant Cell Environ* 15:633–643
- Zimmermann MH (1983) *Xylem structure and the ascent of sap*. Springer, Berlin Heidelberg New York
- Zimmermann MH, Jeje AA (1981) Vessel-length distribution in stems of some American woody plants. *Can J Bot* 59:1882–1892
- Zwieniecki MA, Hutyra L, Thompson MV, Holbrook NM (2000) Dynamic changes in petiole conductivity in red maple (*Acer rubra* L.), tulip tree (*Liriodendron tulipifera* L.), and northern fox grape (*Vitis labrusca* L.). *Plant Cell Environ* 23:407–414