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MECHANISMS FOR TOLERATING FREEZE–THAW STRESS OF TWO EVERGREEN CHAPARRAL SPECIES: *RHUS OVATA* AND *MALOSMA LAURINA* (ANACARDIACEAE)[1]

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The response to freeze–thaw stress was examined for two co-occurring evergreen species, *Malosma laurina* and *Rhus ovata*. Laboratory and field experiments on adults and seedlings were made in the spring and winter in 1996 and again on adults in 2003 and 2004. Laboratory and field results indicated that the stem xylem for adults of *M. laurina* and *R. ovata* were similarly susceptible to freezing-induced cavitation (percentage loss of conductivity = 92 ± 2.6% for *R. ovata* and 90 ± 4.2% for *M. laurina* at ≤ −6°C). In contrast, leaves of *M. laurina* were more susceptible to freezing injury than leaves of *R. ovata*. Among seedlings in the field, leaves of *M. laurina* exhibited freezing injury at −4°C and total shoot mortality at −7.2°C, whereas co-occurring seedlings of *R. ovata* were uninjured. Surprisingly, *R. ovata* tolerates high levels of freezing-induced xylem embolism in the field, an apparently rare condition among evergreen plants. *Rhus ovata* avoids desiccation when xylem embolism is high by exhibiting low minimum leaf conductance compared to *M. laurina*. These results suggest a link between minimum leaf conductance and stem hydraulics as a mechanism permitting the persistence of an evergreen leaf habit in freezing environments.

Key words: Anacardiaceae; California; cavitation; chaparral; embolism; *Malosma laurina*; *Rhus ovata*; xylem.

Freezing survival of evergreen plants involves both the tolerance of the living tissues of the leaves and the function of the nonliving water transport system of the stems. For leaves or living cells, damage and subsequent death can occur when the water in living cells freezes or when cells dehydrate due to the extracellular freezing of water (Sakai and Larcher, 1987). For stems, when sap in the xylem freezes, the gas dissolved in the sap comes out of solution because the solubility of gas is greatly reduced in ice compared to liquid sap. This can lead to the formation of emboli in xylem cells upon thawing if the bubbles do not go back into solution but instead expand in a process called cavitation (Yang and Tyree, 1992). The likelihood that bubbles go back into solution when the sap thaws is greater for smaller bubbles and for plants with xylem sap under less tension (Yang and Tyree, 1992; Langan et al., 1997). In the absence of water stress, woody plants that have mean hydraulic vessel and tracheid diameters that are <35 μm are relatively resistant to cavitation caused by freeze–thaw stress, whereas stems that have hydraulic mean vessel and tracheid diameters >35 μm are susceptible to such cavitation (Davis et al., 1999; Pittermann and Sperry, 2003).

For an evergreen woody plant, if the xylem transport system is rendered permanently nonconductive due to cavitation caused by freezing, then tolerance of the leaves to freezing becomes moot because the leaves will desiccate without an adequate supply of water. Because of this, the stem xylem should be greater than or equal in its resistance to cavitation caused by freeze–thaw stress compared to the degree of freeze–thaw stress damaging leaves. Consistent with this model, the leaves of the chaparral shrub *Ceanothus crassifolius* only tolerate freezing temperatures to −18°C, whereas its stems resist severe freeze–thaw embolism when frozen to −25°C at a branchlet water potential (Ψ_x) of −5 MPa (Ewers et al., 2003). Further, both the leaves and stems of the chaparral shrubs *C. megarcarpus* and *C. spinosus* are intolerant of a −10°C freeze–thaw, i.e., the leaves become damaged and the stems become highly embolized (95–100% loss in hydraulic conductivity) when frozen to −10°C and subsequently thawed at a Ψ_x of −5 MPa (Langan et al., 1997; Boorse et al., 1998a; Ewers et al., 2003).

A broad-leaved evergreen plant that does not appear to fit these patterns for chaparral species is *Rhus ovata* S. Watson. This plant commonly occurs in cold-air drainage basins and at higher elevations in the chaparral where subfreezing temperatures are quite common, yet it has relatively large diameter vessels. Our preliminary results indicated that it had vessels as large as 80 μm in diameter. These large vessel diameters are similar to another evergreen shrub, *Malosma laurina* (Nutt.) Brewer and S. Watson, also of the Anacardiaceae (mean vessel diameter = 59.2 μm; Carlquist and Hoekman, 1985). By comparison, Carlquist and Hoekman (1985) found a mean vessel diameter of 29.2 μm, for 41 chaparral species.

Rhus ovata and *M. laurina* share many similarities. For example, they broadly overlap in their distributions in southern California, are evergreen, resprout and recruit from seed after wildfire (facultative sprouting), have similar leaf size and shape, have similar minimum seasonal water potentials (Miller and Poole, 1979), and have similar resistance to cavitation caused by water stress (S. Davis, unpublished data).

However, *M. laurina* and *R. ovata* are not similar in their freezing tolerance. *Malosma laurina* is highly vulnerable to freeze–thaw embolism and is restricted to nonfreezing sites in the chaparral and coastal sage scrub of southern California (Langan et al., 1997). *Rhus ovata* shows elevated native embolism at freezing sites, yet it persists with no visible damage

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to its leaves or stems at sites where winter low temperatures can reach −12°C (Langan et al., 1997). This presents an interesting paradox because stems of *R. ovata* are highly vulnerable to cavitation caused by freeze–thaw stress, whereas its leaves apparently tolerate freezing temperatures and do not desiccate when the stems are highly embolized.

Here we examined the response to freeze–thaw stress for two chaparral species, *M. laurina* and *R. ovata*, that have overlapping distributions in southern California. We hypothesized that, for *R. ovata*, the stems would experience high levels of embolism following freeze–thaw, whereas the leaves would not suffer damage. In contrast, for *M. laurina*, we predicted similar susceptibility to freeze–thaw stress for both stems and leaves. Some have suggested that the parenchyma of stems may be killed by freezing temperatures and that this can lead to cavitation (Pockman and Sperry, 1997; Martinez-Vilalta and Pockman, 2002; Pittermann and Sperry, 2003). To address this possibility, we examined death among ray parenchyma cells following freezing treatments to assess if cavitation caused by freeze–thaw was concurrent with the death of xylem parenchyma. In addition, we examined traits that would allow *R. ovata* to avoid or tolerate cavitation caused by freezing, such as phenology of leaf production and reduced leaf conductance to water vapor. Finally, we present unique data from a natural field experiment causally linking freezing temperatures to differential seedling mortality in *R. ovata* and *M. laurina* growing side by side.

MATERIALS AND METHODS

Plants and study sites—We studied adults and seedlings of two co-occurring chaparral species, *Malosma laurina* (formerly *Rhus laurina*) and *Rhus ovata* (nomenclature follows Hickman, 1993).

We studied at two sites: a warm site near the ocean and a cold site 6 km inland from the coast near Cold Creek Canyon (described in Langan et al., 1997 and Ewers et al., 2003). The lowest temperature reported at the cold site is about −12°C, and temperatures regularly reach −8°C for a seasonal low (Ewers et al., 2003). We monitored temperature during the study with a max–min thermometer (McMaster Carr, Los Angeles, California, USA), and previous studies have systematically documented temperatures throughout the site (Langan et al., 1997). The warm site was located in the city of Malibu near Pepperdine University. This site served as a nonfreezing control site for experiments with *M. laurina* seedlings. The minimum temperature at this site rarely reaches 0°C due to its proximity to the Pacific Ocean. We monitored temperature and rainfall at this site with a max–min thermometer and manual rain gauge (All Weather Rain Gauge, Productive Alternative Inc., Fergus Falls, Minnesota, USA).

At our cold study site, adults of *M. laurina* and *R. ovata* are differentially distributed, whereas seedlings of the two species can co-occur following wildfire. At this site, adults of *M. laurina* are restricted to ridge crests that remain 3–4°C warmer than valley bottoms where cold air drainage is common (Langan et al., 1997; Ewers et al., 2003). Severe freezing events every 10 to 20 yr can cause total shoot dieback of *M. laurina* adults (−11°C in 1990; Boorse et al., 1998b; Fig. 2A), yet *M. laurina* can persist along the ridge crests at the site by resprouting from a large root crown (DeSouza et al., 1986; Frazer and Davis, 1988; Thomas and Davis, 1989). *Rhus ovata* occurs throughout the site and overlaps in its distribution with *M. laurina* along the ridge crests. Adults of *M. laurina* were never observed in the cold-air drainages of valley bottoms, but seedlings emerged in the months following a wildfire on 21 October 1996 and persisted throughout the summer and fall of 1997. Presumably, the source of the seedlings was the adults along the ridge crests, and their germination was promoted by the wildfire. *Rhus ovata* seedlings also germinated after the wildfire in the valley bottoms where they co-occurred with *M. laurina* seedlings.

The wildfire of 1996 also burned a mixed stand of chaparral at a site in Malibu that does not experience freezing temperatures in the winter months. Following this fire, *M. laurina* seedlings emerged at this nonfreezing site. This pattern of burn and seedling emergence facilitated a natural experiment for us to compare same-aged seedlings at a freezing and a nonfreezing site.

Response of branches and leaves to freezing and thawing—For adults, response to freezing temperatures was evaluated by freezing branches in a cooling chamber (described in Langan et al., 1997) in May and June 1996 (springtime) and again in January and February 2003 (wintertime). Cooling rate was controlled at −0.08°C/min between 15 and 0°C and a rate of −0.02°C/min between 0 and −10°C. Warming between −10 and 15°C occurred at a rate of 0.08°C/min. These cooling and thawing rates were chosen because they matched the rates measured on leaves and stems of *M. laurina* and *R. ovata* in the field at our cold study site (Langan et al., 1997).

For treatment in the cooling chamber, large branches (≥1 m in length) were removed from plants in the field at predawn, double-bagged, and brought back to the laboratory in ≤30 min. In the laboratory, excised branches were recut under water and left to sit with their excised ends submerged in water for about 2 h. Before and after rehydration, branchlet water potential (Ψ_x) was estimated on two to four terminal branches using a pressure chamber (PMS, Corvalis, Oregon, USA). After hydration, a branch was inserted into the cooling chamber and a freeze–thaw cycle was begun. Temperature of the xylem was estimated by inserting a thermocouple just under the bark on a side branch, and as a control, a thermocouple was attached to a dowel rod. A second branch was bagged and left in an air-conditioned laboratory for the duration of the freeze–thaw treatment, serving as an unfrozen control. Both the control and frozen branches were kept hydrated by maintaining their excised ends in a bucket of water throughout the experiment. After the freeze–thaw treatment, Ψ_x was again estimated for the treated and control branches.

The amount of embolism caused by the freeze–thaw treatment was estimated as the percentage loss of hydraulic conductivity (PLC) following the freeze–thaw in comparison to the unfrozen control. Following the freeze–thaw, no less than three lateral stems (about 14 cm long) were removed from both the treated and control branches by cutting them under water. For these stems, hydraulic conductivity (K_h; kg · m · MPa^{-1} · s^{-1}) was measured by perfusing ultrafiltered (0.1 μm pore filter) de-gassed acid solution through them at low pressure (~2 kPa; Sperry et al., 1988; Stiller and Sperry, 2001). A low pH solution (pH 2, HCl) was used to prevent microbial growth (Sperry et al., 1988). Following initial measurement of K_h, stems were flushed with acid solution at about 100 kPa for ≥60 min. The post-flushing K_h represented maximum K_h (K_{hmax}), i.e., K_h with no emboli in the xylem. Percentage loss of hydraulic conductivity (PLC) was calculated as $(1 - K_h/K_{hmax}) \times 100$.

Freezing damage to living cells of leaves and stems was assessed. For leaves, damage was assessed using dark-adapted fluorescence (F_v/F_m) measured before and after the freeze–thaw treatments. Chlorophyll fluorescence from photosystem II is a fast and reliable indicator of low temperature stress of chloroplasts (Larcher, 1995; Loik and Redar, 2003; Percival and Henderson, 2003). In addition, previous work has found that estimating leaf damage following freezing using F_v/F_m for *M. laurina* and *R. ovata* gave similar results to three other methods including electrolyte leakage (Boorse et al., 1998b). Following the freeze–thaw, cuvettes were attached to six leaves of the treated and control branches to dark-adapt them for 20 min before F_v/F_m was measured using a pulse-modulated fluorometer (Opti-Sciences, OS1-FL, Tyngsboro, Massachusetts, USA).

For stems, the vitality of parenchyma cells was estimated using the fluorescent stain fluorescein diacetate (Rotman and Papermaster, 1966; Windholm, 1972; Boorse et al., 1998b). In a viable cell, the relatively nonpolar and nonfluorescent fluorescein diacetate diffuses across the cell membrane where it is hydrolyzed by esterases into the more polar and fluorescent fluorescein. If the cell membrane is intact and the esterases are functional, the fluorescein will accumulate in the cell faster than it leaks out and fluoresce green when excited by blue actinic light (Rotman and Papermaster, 1966). If the esterases are not functional or the membrane is damaged then the fluorescein will not form and accumulate in the cell, and florescence will not occur.

Control and freeze–thaw stems were thin-sectioned and placed in 10 mL of deionized water to which 0.1 mL of 0.01% fluorescein diacetate in acetone

was added. After 1 min, another 10 mL of deionized water was added to the solution. Sections were incubated in the solution for about 10 min for *R. ovata* and 60 min for *M. laurina* to allow the dye to infiltrate the cells. In preliminary trials, the dye infiltrated more slowly into *M. laurina* than into cells of *R. ovata*, necessitating a longer incubation time for *M. laurina*. After the incubation, sections were mounted in deionized water and viewed under blue fluorescent light (excitation filter BG-12) using an epifluorescence microscope (Nikon, Garden City, New York, USA) at 200× magnification. Control stems were sectioned and stained the same as treated stems. In addition, a second control stem was submerged in liquid nitrogen for about 10 s, thawed at room temperature, and stained. This second control verified that fluorescence was not present when cells were dead.

The injury of leaves in response to cooling temperatures was estimated in three ways during March and April 1996. Branches were frozen in a cooling chamber (as described earlier) to increasingly negative temperatures. For every 1°C decrease in temperature below 0°C, leaves were carefully removed from branches with clippers attached to a meter stick so as to not alter the temperature in the cooling chamber. Leaves were immediately placed in a sealed container in an ice chest to facilitate gradual thawing overnight. The lethal temperature at 50% cell death or damage (LT_{50}) was estimated using F_v/F_m, vital staining with fluorescein diacetate, and a color change score using the Munsel color chart (1977 ed., Forestry Supply, Jackson, Mississippi, USA). All of the techniques to estimate the LT_{50} for leaves generally agreed within 1°C and are described in detail in Boorse et al. (1998b).

The effect of freezing on stem conductivity for *R. ovata* was also estimated on 21 January 2004 by measuring PLC for plants growing in the field following four subzero temperature events of ≤−6°C in December 2003, with the lowest being −7.5°C on 19 December 2003. For these measurements, stems were removed from seven plants. All cuts to stems were made under water to prevent air from entering the xylem. Once removed, stems were double bagged and transported to the laboratory in <60 min. In the laboratory, stems were recut under water to 10 cm. The PLC was measured as described and represents an estimate of native-state embolism.

Experiments with seedlings—We sampled seedlings of *M. laurina* and *R. ovata* that germinated after a wildfire on 21 October 1996. Following this fire, the cold site was surveyed, and all *M. laurina* seedlings were tagged, mapped, and followed over time. All seedlings at our cold study site were found in the cold valley bottom. Apparently, the seedlings that germinated after the wildfire did so after the coldest part of the winter, which is typical for *M. laurina*. That is, *M. laurina* typically germinates in January and February (Thomas and Davis, 1989), and December is often the month with the minimum temperature for the season at our cold study site (Langan et al., 1997). About 7% of the *M. laurina* seedlings survived the subsequent dry season in 1997, and these are the seedlings we sampled in December 1997. At this time, we located co-occurring *R. ovata* seedlings for comparison. We measured F_v/F_m and survivorship for 12 seedlings of *M. laurina* and *R. ovata* following freezing and subfreezing temperatures. In addition to the 12 *M. laurina* seedlings at the cold site, as a control, F_v/F_m was measured for 10 seedlings of *M. laurina* at a site about 5 km away that did not have freezing temperatures because it is near the Pacific Ocean. Seedlings from the warm and cold site were approximately the same age because they all germinated after the Calabasas/Malibu wildfire of 1996.

Leaf temperature of seedlings at the cold and warm sites was monitored continuously in December 1997. Abaxial leaf temperature was measured with a thermistor and datalogger (Onset, Pocasset, Massachusetts, USA).

Vessel diameter—Vessel diameters were measured for stem segments exposed to freezing temperatures in the cooling chamber. Transverse sections were made at the midpoint of ~6 mm diameter stem segments with a microtome (American Optical Company, Buffalo, New York, USA), and vessel diameters were measured in four nonoverlapping areas (two near the pith and two near the stem surface), using a compound microscope (Nikon) with a digital camera (Spot RT Color Camera, Diagnostic Instruments Inc., Sterling Heights, Michigan, USA) and imaging software (ScionImage version Beta 3b, Scion Corporation, Frederick, Maryland, USA). The number of vessels measured per cross section ranged from 65 to 155, and a pooled mean was calculated for each species. We also expressed vessel diameters in terms of mean hydraulic vessel diameter, $\Sigma d^5/\Sigma d^4$, where d = vessel diameter (Sperry et al., 1994).

Minimum leaf conductance in the laboratory—We hypothesized that to tolerate high levels of stem embolism in a field environment after freeze–thaw stress, *R. ovata* would have a lower minimum leaf conductance compared to *M. laurina*. Transpiration and whole leaf conductance (stomatal and cuticular) were assessed for excised leaves of both species in June and July 2003 and February 2004. Because results from each of the three sampling dates were similar, data are only presented from July 2003 and February 2004. Six branches (≥1 m in length) were removed from plants at our cold study site in Cold Creek Canyon, immediately bagged and returned to the laboratory in ≤30 min. Stems were then recut under water and left to sit with their excised ends submersed in water for about 12 h. After hydration, inside a humid chamber, two mature leaves were removed from each branch and labeled, and cut surfaces of petioles were covered with fast-drying epoxy (Quick Set Epoxy, Loctite, Rocky Hill, Connecticut, USA) to prevent water loss from cut surfaces. Upon removing leaves from the humid chamber, their mass was measured, with a 0.01 mg resolution analytical balance (AE163, Mettler-Toledo, Columbus, Ohio, USA), as was dark-adapted F_v/F_m. Leaves were then placed on a mesh screen on a rack suspended about 10 cm above a laboratory bench top in June and July and in a controlled environment chamber in February. Light intensity was low: photosynthetically active radiation level for experiments in June and July 2003 was 12 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and in February 2004 it was at 55 $\mu mol \cdot m^{-2} \cdot s^{-1}$. In the June, July, and February experiments, the vapor pressure deficit ranges were 1.4–2.3, 0.5–1.2, and 1.2–1.9, respectively, and the air temperature ranges were 21–22°C, 20–25°C, and 21–22°C, respectively. The mass and F_v/F_m were measured 30 min after the initial measurement and for every hour after that for the first 5 h. Measurements were then made every 1 to 8 h until the mass remained constant between measurements and F_v/F_m was not different from 0. The dew-point temperature (T_d) (Hygrometer, Edge Tech, Milford, Massachusetts, USA) and the air temperature adjacent to leaves (T_a) were measured each time the leaf mass was measured. Vapor pressure deficit (VPD) between the leaves and the air was calculated as $e_{sat}(T_d) - e_{sat}(T_a)$, where e_{sat} is the saturation vapor pressure (kPa) (Campbell and Norman, 1998). Measurements indicated that the leaf temperature was not different from the air temperature. Whole leaf conductance (g_{leaf}; $mmol \cdot m^{-2} \cdot s^{-1}$) was calculated from the following relationship:

$$g_{leaf} = E/(VPD/P_a),$$

where E is the rate of water loss divided by the leaf area times two, i.e., transpiration (Kerstiens, 1996) and P_a is atmospheric pressure (102.28 kPa in Malibu, California). The leaf area was multiplied by two because water was being lost from both leaf surfaces, a common practice when estimating g_{leaf} (Kerstiens, 1996). The area of the leaves was measured with a leaf area meter (Li-3100, Li-Cor Inc., Lincoln, Nebraska, USA).

Diurnal measurements in a field environment—To determine if *R. ovata* had low g_s and was under water stress resulting from high levels of embolism, diurnal g_s and Ψ_x were measured on *R. ovata* in the valley bottom and on *M. laurina* along the ridge crest immediately upslope from *R. ovata* at our cold study site on 6 February 2004. Stomatal conductance was measured with a porometer (Li-1600, Li Cor Inc.). Water potential was measured with a pressure chamber on branchlets in the field (PMS). Both g_s and Ψ_x were measured every 2 or 3 h from predawn hours until dusk with one exception; we could not measure g_s at predawn because the temperature was below freezing, preventing porometer function within manufacturer's specifications.

Tissue water relations—To assess if osmotic adjustments or tissue capacitance could explain the differential freezing tolerance between *R. ovata* and *M. laurina*, we estimated the capacitance and tissue water relations for leaves of both species using a pressure-volume technique modified from Tyree and Hammel (1972). Twelve branches, about 25 cm long, from six individual plants of each species were cut under water at 1800 hours on 12 May 2004.

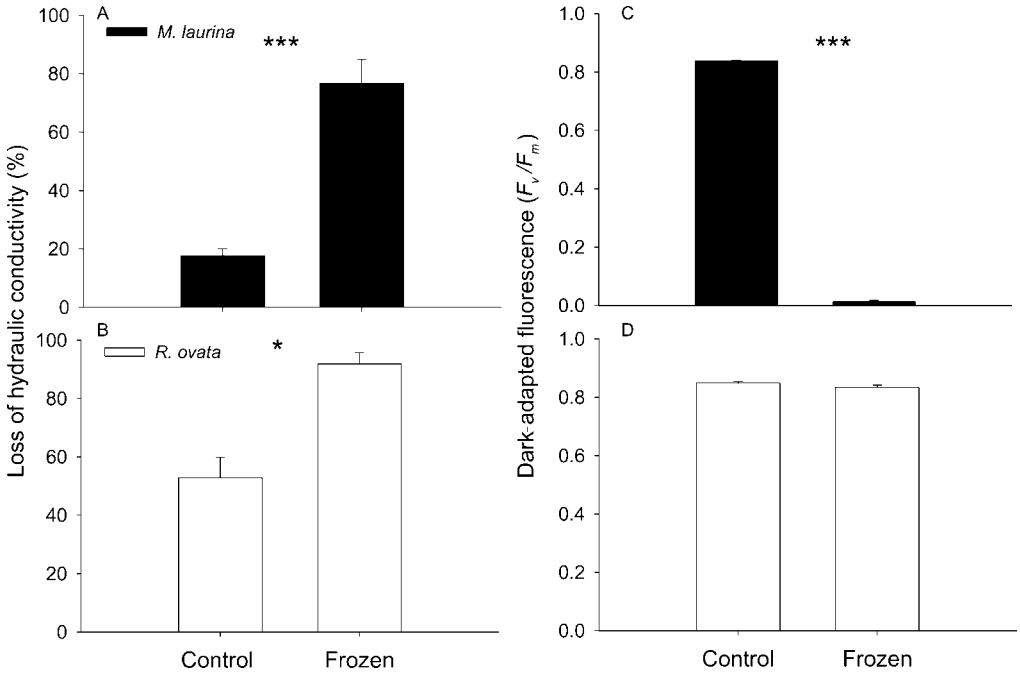

Fig. 1. Loss of hydraulic conductivity for stems (A and B) and decline in dark-adapted fluorescence for leaves (C and D) displayed for *Malosma laurina* and *Rhus ovata* following a freeze of −10°C. Large branches (≥1 m) were hydrated (prefreeze branchlet Ψ_x = −0.70 MPa for *M. laurina* and −0.97 MPa for *R. ovata*) and frozen in a cooling chamber. Bars represent means ±1 SE (N = 3 for *M. laurina* and N = 6 for *R. ovata*). Asterisks indicate differences between frozen and control stems (* = $P < 0.05$, and *** = $P < 0.001$). Data were collected in January and February 2003.

The cut end of each branch was immediately submerged in water, bagged, and allowed to rehydrate for 12 h for *R. ovata* and 24 h for *M. laurina*. "Plateau effects" described by Kubiske and Abrams (1991), indicating the filling of airspaces in leaves during saturation, were not apparent. Parameters for tissue water relations were analyzed and calculated following Koide et al. (1989).

Statistics—Data were analyzed using *t* tests and ANOVAs (Minitab v.14.12, Minitab, Inc., State College, Pennsylvania, USA), and transformed as necessary to satisfy assumptions of statistical models. When comparing treatments, differences were considered significantly different at alpha ≤ 0.05. If P was ≥ 0.05, we reported comparisons as similar or not different.

RESULTS

Percentage loss of hydraulic conductivity (PLC) of hydrated stems did not differ between *R. ovata* and *M. laurina* when exposed to freeze–thaw stress. Among individuals sampled in May and June 1996, PLC for stems frozen to −6°C was greater than 92% for both species, and greater than that for control stems (data not shown). Branchlet water potentials (Ψ_x) for stems frozen to −6°C were −0.66 MPa for *M. laurina* and −0.55 MPa for *R. ovata*. The Ψ_x for control and frozen stems were not different. Additional experiments performed in January and February 2003 again showed that the xylem of *M. laurina* and *R. ovata* exhibited similar PLC when exposed to freezing temperatures (Fig. 1A, B). For both species, PLC for stems frozen to −10°C was greater than that of unfrozen control stems. Branchlet water potential for treated stems was not different from that for control stems for both genotypes: Ψ_x = −0.70 MPa for *M. laurina* and −0.97 MPa for *R. ovata*. To verify laboratory experiments with *R. ovata*, native-state PLC and leaf-specific conductivity (K_l) were measured on 21 January 2004 following multiple freeze–thaw events in December 2003 and January 2004. At this time, PLC and K_l were 92 ± 1.9% and 0.45 ± 0.16 × 10^{-4} kg · s^{-1} · MPa^{-1} · m^{-1}, respectively, and predawn Ψ_x was −1.88 ± 0.09 MPa. For *M. laurina* along the ridge crests on 3 March 2004, PLC was 70 ± 5.0% and K_l was 2.37 ± 0.35 × 10^{-4} kg · s^{-1} · MPa^{-1} · m^{-1}.

In contrast to the stems, the leaves of *M. laurina* were more susceptible to freezing injury and death than the leaves of *R. ovata*. For experiments in the early spring, March and April 1996, the lethal temperature for 50% cell death (LT_{50}) was −5.7°C for *M. laurina* and −10.0°C for *R. ovata*. The results were not different when LT_{50} was estimated as cell death (indicated with vital stain), 50% reduction in F_v/F_m, or relative color change. For experiments in midwinter, January and February 2002, for *M. laurina*, freezing leaves to −10°C reduced F_v/F_m ($F_v/F_m \cong 0$) compared to unfrozen control leaves, whereas for *R. ovata*, the F_v/F_m for frozen and unfrozen leaves did not differ (Fig. 1C, D).

The ray parenchyma of stems for both species appeared to survive freezing temperatures of −10°C (Fig. 2C, G). Vitally stained stem parenchyma cells exposed to −10°C fluoresced similarly to cells of unfrozen control stems (Fig. 2C, D, G, H). In other words, freezing to −10°C caused no detectable cell death in stems of either species. The −80°C control did not fluoresce, indicating that our fluorescein diacetate stain was not causing fluorescence in nonliving cells (Fig. 2B and F).

The vessel diameters for *M. laurina* and *R. ovata* were not different (Fig. 3). For example, the mean, the hydraulic mean, and the maximum vessel diameters for *M. laurina* (27.4 ± 3.2, 46.8 ± 6.0, and 64.2 ± 7.3 μm, respectively) were not different from those of *R. ovata* (25.9 ± 1.4, 40.3 ± 2.6, and 55.4 ± 3.2 μm, respectively).

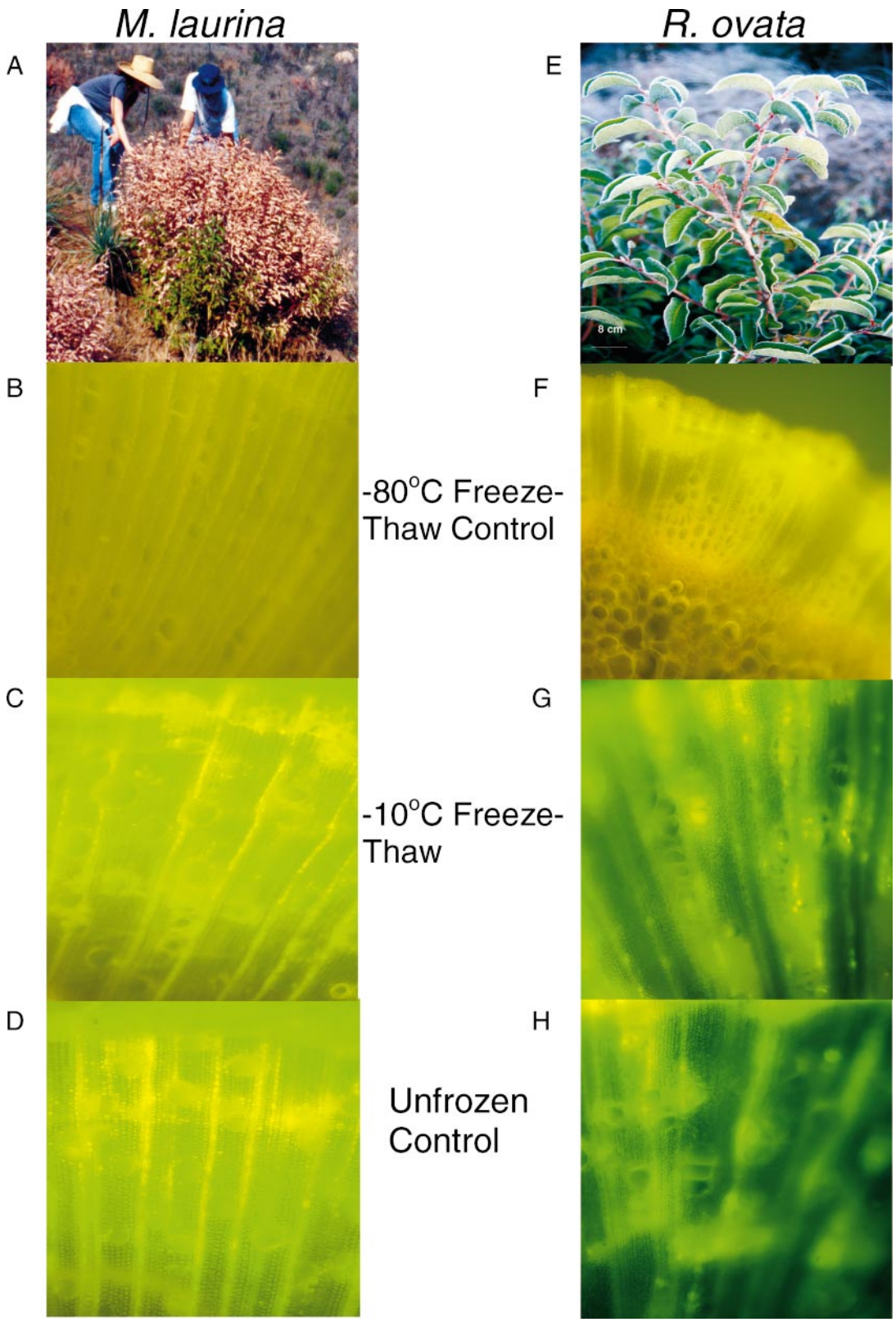

Fig. 2. Photographs of *Malosma laurina* (A-D) and *Rhus ovata* (E-H) showing their differential tolerance of freeze–thaw stress. Near our cold study site, following freeze–thaw stress, *M. laurina* shows widespread shoot death (A); in contrast, *R. ovata* tolerates freeze–thaw stress and is shown with ice crystals on its evergreen leaves (E). In addition, representative xylem cross-sections are displayed for stems of *M. laurina* (B, C, and D) and *R. ovata* (F, G, and H) following a freeze of −10°C (C and G) and for unfrozen control stems (D and H). Sections are stained with fluorescein diacetate to assess the viability of xylem parenchyma (see Materials and Methods). Stems for −80°C control were submerged in liquid nitrogen for 10 s to kill stem parenchyma cells (B and F). Data were collected during the winter months of January and February 2003. Magnification is 200× for cross-sections.

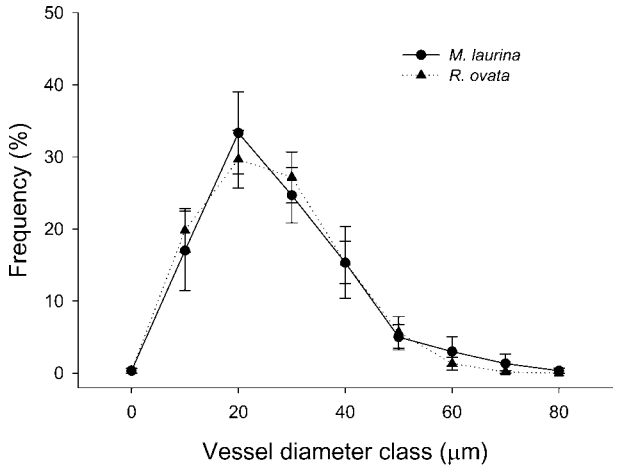

Fig. 3. Frequency of vessel diameters displayed for *Malosma laurina* and *Rhus ovata*. Data points represent means ±1 SE ($N = 3$ for *M. laurina* and $N = 6$ for *R. ovata*).

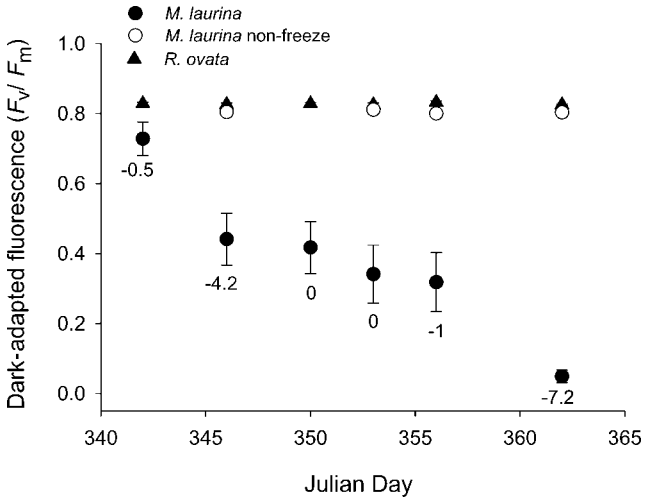

Fig. 4. Dark-adapted fluorescence in leaves of naturally occurring seedlings following zero and subzero temperatures for *Malosma laurina* and *Rhus ovata* in December 1997. Data are shown for seedlings of *M. laurina* co-occurring with *R. ovata* at a site where freezing occurs (closed circles and closed triangles, respectively) and for *M. laurina* seedlings growing at a site with no freezing (open circles). The previous night's minimum temperature for the freezing site is shown below the closed circles. Data points represent means ±1 SE ($N = 10$–12). The *M. laurina* seedlings at the cold site showed 100% mortality following the −7.2°C temperature, whereas the co-occurring *R. ovata* seedlings showed no mortality.

Naturally occurring seedlings of *M. laurina*, about 1 year old, had reduced dark-adapted F_v/F_m during subzero temperatures of −0.5 and −4.2°C (Fig. 4). Further, these seedlings exhibited F_v/F_m of about 0 after a freeze of −7.2°C, and the entire shoot of all seedlings died in December 1997 (Fig. 4). The apparently dead seedlings were surveyed repeatedly for 12 months and were verified to be dead. As a control, *M. laurina* seedlings at a nonfreezing site showed no reduction in F_v/F_m over the same time period where the minimum temperature was +6.7°C (Fig. 4). In contrast to *M. laurina* seedlings at the freezesite, co-occurring *R. ovata* seedlings showed no reduction in F_v/F_m following subzero temperatures (Fig. 4), and none of the *R. ovata* seedlings were killed.

Transpiration (E) and conductance of whole leaves (g_{leaf}) were greater in *M. laurina* compared to *R. ovata* during benchtop, dry-down experiments in July 2003 and February 2004. Over the first 24 h of the experiment in July, the average g_{leaf} of *M. laurina* was twice that of *R. ovata* (10.40 0.91 and 5.68 ± 0.79 mmol · m^{-2} · s^{-1}, respectively; $P < 0.01$; Fig. 5A), whereas the rate of decline in g_{leaf} was not different between the two species (rate of g_{leaf} decline = $-4.94 \pm 0.15 \times 10^{-4}$ mmol · m^{-2} · s^{-2} for *M. laurina* and $-3.22 \pm 0.85 \times 10^{-4}$ mmol · m^{-2} · s^{-2} for *R. ovata*; $P = 0.36$). Over this same time interval, the average rate of E for leaves was also about twofold greater in *M. laurina* compared to *R. ovata* ($1.70 \pm 0.16 \times 10^{-6}$ and $0.90 \pm 0.14 \times 10^{-6}$ kg · m^{-2} · s^{-1}, respectively; $P < 0.01$; Fig. 5B). Dark-adapted fluorescence showed an accelerated decline for *M. laurina* compared to *R. ovata*. The time it took for F_v/F_m to decline by 50% was about four times faster for *M. laurina* compared to *R. ovata* (28.3 ± 9.4 and 104.7 ± 10 h, respectively; $P < 0.001$; Fig. 5C). Results in the winter, February 2004, were similar to those in July 2003. Over the first 24 h of the experiment in February, the average g_{leaf} of *M. laurina* was twofold greater compared to *R. ovata* (6.05 ± 0.61 and 3.03 ± 0.31 mmol · m^{-2} · s^{-1}, respectively; $P < 0.01$; Fig. 5F), whereas the rate of decline in g_{leaf} was not significantly different between the two species (rate of g_{leaf} decline $-1.12 \pm 0.26 \times 10^{-4}$ mmol · m^{-2} · s^{-2} for *M. laurina* and $-0.66 \pm 0.26 \times 10^{-4}$ mmol · m^{-2} · s^{-2} for *R. ovata*; $P = 0.25$). Transpiration of leaves was also about twofold greater for *M. laurina* compared to *R. ovata* ($1.96 \pm 0.20 \times 10^{-6}$ and $0.89 \pm 0.04 \times 10^{-6}$ kg · m^{-2} · s^{-1}, respectively; $P <$ 0.01; Fig. 5G). The time it took for F_v/F_m to decline by 50% was about four times faster for *M. laurina* compared to *R. ovata* (14.0 ± 1.8 and 59.7 ± 6.3 h, respectively; $P < 0.01$; Fig. 5H). The relative water content (RWC) and leaf Ψ_x are also shown during the dry-down experiment (Fig. 5D, I, E, J, respectively). These values were estimated from pressure-volume curves of leaves for the two species. After 24 h, for experiments in July and February, the g_{leaf} for the two species did not differ, but by this time *M. laurina* leaves appeared to be depleted of water, based on the leveling of the water loss per unit leaf area and the RWC.

Tissue water relations, estimated using pressure-volume curves of mature leaves, did not differ between *M. laurina* and *R. ovata* (see Table 1).

Diurnal g_s and Ψ_x for *M. laurina* and *R. ovata* measured at our cold study site on 6 February 2004 showed that g_s and Ψ_x were greater for *M. laurina* compared to *R. ovata* (Fig. 6A, B). Branchlet water potential was more than two times lower for *R. ovata* compared to *M. laurina* for all hours of the day (Fig. 6A). Stomatal conductance for *R. ovata* was about half that for *M. laurina* at 0800, 1515, and 1700 hr (Fig. 6B). At 1015 and 1315 hr, g_s was not different between the two species (Fig. 6B). The day was clear and sunny, with photosynthetically active radiation peaking at 1200 hours at 1500 μmol · m^{-2} · s^{-1} (Fig. 6C). Low temperature overnight (between 5 and 6 February) was −4°C, and the high temperature on 6 February was 23.6°C (data not shown).

Predawn Ψ_x and the timing of new leaf growth were different between *M. laurina* and *R. ovata* at our cold study site in the winter months of 2004 (Fig. 7A, B). Branchlet water potentials measured at predawn (Ψ_{pd}) were lower in February, March, and April 2004 for *R. ovata* compared to *M. laurina* (Fig. 7A). In June (Julian days 153–182), Ψ_{pd} for *R. ovata* and *M. laurina* converged and were no longer different (Fig. 7A).

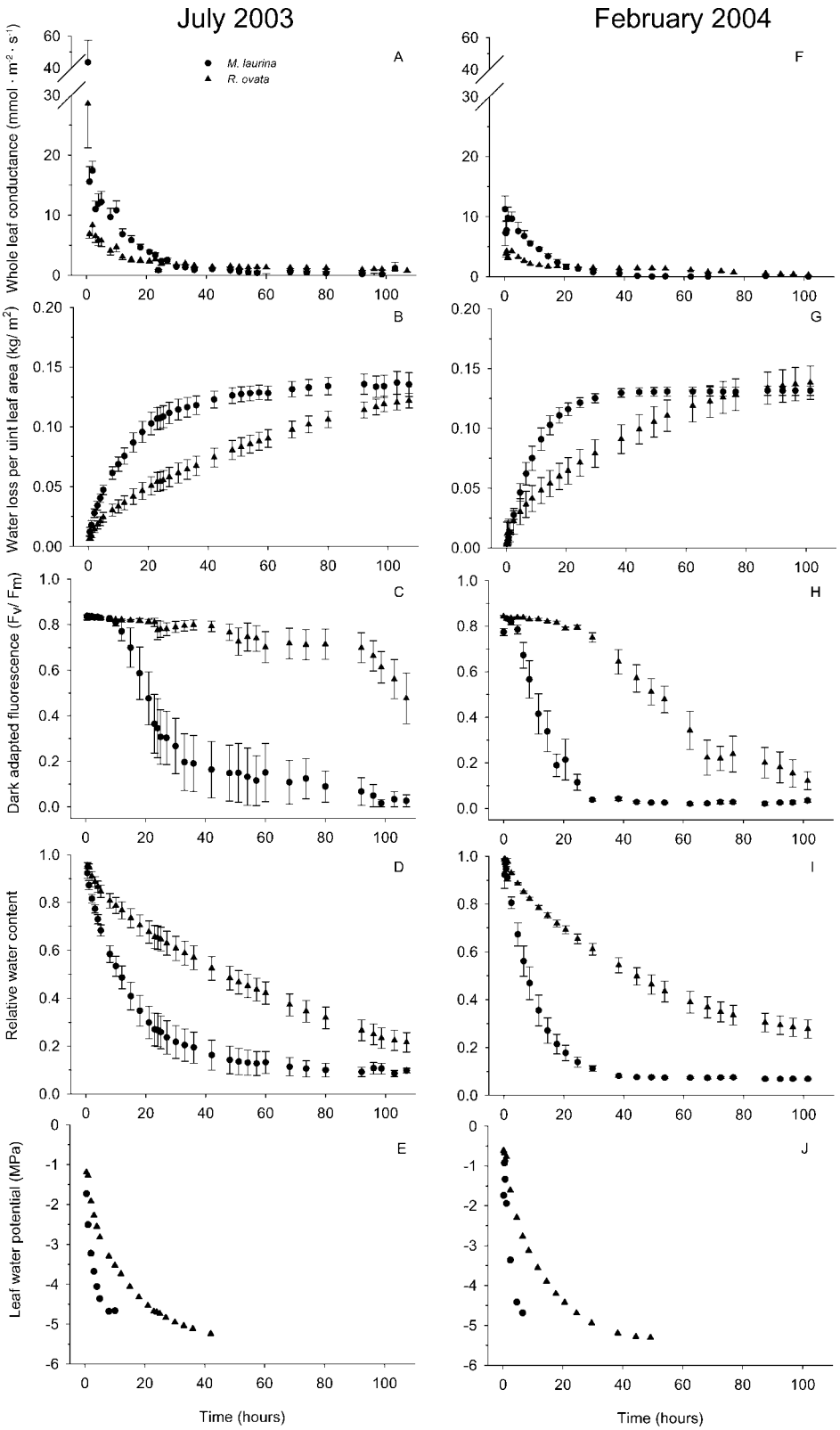

Fig. 5. Whole leaf conductance (stomatal and cuticular; A and F), cumulative water loss per unit leaf area (B and G), dark adapted F_v/F_m (C and H), relative water content (D and I), and leaf water potential (E and J) for *Malosma laurina* and *Rhus ovata*. Data were collected on excised leaves in a laboratory in three separate experiments in June and July 2003 and February 2004. Results were similar between the three sampling dates, and data shown are from July 2003 and February 2004 only. Data points represent means ±1 SE ($N = 6$). Leaf water potentials were estimated from a polynomial equation calculated from the relationship between water potential and relative water content; therefore, there are no error bars. The average vapor pressure deficit ±1 SD during dry downs was 0.87 ± 0.12 in July and 1.81 ± 0.07 in February.

TABLE 1. Tissue capacitance (C), bulk modulus of elasticity near saturation (ϵ), the leaf water potential at the turgor loss point (Ψ_{tlp}), the relative water content at the turgor loss point (RWC_{tlp}), the bound or apoplastic relative water content (B), and the osmotic potential for saturated tissue $\Psi_{s(sat)}$ displayed for two species, *Malosma laurina* and *Rhus ovata*. Data points represent means ±1 SE ($N = 5$ for *M. laurina* and 6 for *R. ovata*). The two species did not differ among any of the parameters ($P > 0.05$).

Species	C (ΔRWC/ΔMPa)	ϵ (MPa^{-1})	Ψ_{tlp} (MPa)	RWC_{tlp} (g/g)	B (g/g)	$\Psi_{s(sat)}$ (MPa)
M. laurina	0.04 ± 0.004	11.8 ± 0.98	−2.27 ± 0.18	0.91 ± 0.01	0.41 ± 0.04	−2.04 ± 0.09
R. ovata	0.04 ± 0.003	10.1 ± 0.74	−2.04 ± 0.08	0.91 ± 0.01	0.42 ± 0.03	−1.92 ± 0.04

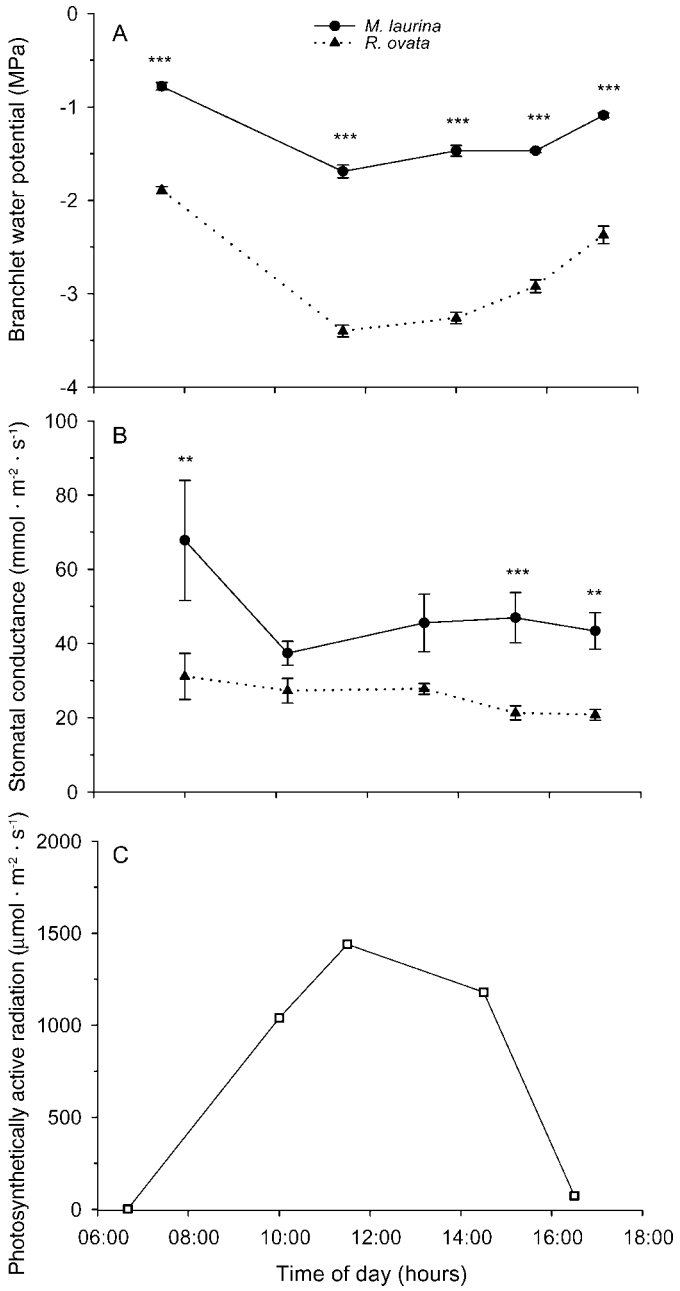

Fig. 6. Diurnal changes in branchlet water potential (A), stomatal conductance (B), and PAR (C) for *Malosma laurina* and *Rhus ovata*. Data were collected on adult plants growing at our cold field site on 6 February 2004. The temperature at predawn (about 0600 hours) was −4°C and the high for the day was 23.6°C at 1630 hr. Data points represent means ±1 SE ($N = 10$). Asterisks indicate significant differences between *M. laurina* and *R. ovata* (** = $P < 0.01$; *** = $P < 0.001$).

The timing of growth for new leaves occurred about two months later in the season for *R. ovata* compared to *M. laurina* (Fig. 7B). Some plants of *M. laurina* initiated new leaves in early March (Julian day 70), whereas *R. ovata* did not begin growth until early May (Julian day 130) (Fig. 7B). All plants of *M. laurina* initiated new leaves by early April and by late May for *R. ovata* (Fig. 7B). To put these measurements into context, December, January, and February were the coldest months, with at least 10 nights with lows of −4°C or lower, with a minimum of −7.5°C on 19 December 2003 (Fig. 7C; data for December is not shown). No subzero temperatures were recorded after 24 April 2004 (Julian day 115). Precipitation from January through March 2004 is shown in Fig. 7D.

DISCUSSION

The freezing results for *R. ovata* present an interesting paradox. As predicted from their large vessel diameters, stems for *R. ovata* and *M. laurina* experienced over 90% loss in hydraulic conductivity at temperatures of ≤6°C. At first glance, this result would seem paradoxical for *R. ovata*, because it is evergreen and presumably the leaves will desiccate if the xylem does not supply them with water year round. For example, a co-occurring evergreen chaparral species, *Ceanothus crassifolius*, undergoes dieback of side branches when stems are highly embolized (Davis et al., 2002). *Malosma laurina* similarly had more than a 90% loss in hydraulic conductivity, but leaf desiccation is not a factor because freezing temperatures of ≤−6°C caused both leaf and stem death for this species (Fig. 2A; Langan et al., 1997). Besides our cold site, *R. ovata* occurs at inland sites in California and Arizona at elevations up to 1300 m (Hickman, 1993), where frosts of ≤−6°C are common in the winter. Maintaining water flow to leaves may be especially important during winter months in southern California, where a night of subzero temperatures may be followed by a warm, sunny day. We conclude that *R. ovata* can tolerate high levels of embolism while maintaining high leaf area (Fig. 2E), a combination that is apparently rare among evergreen woody plants, based on available data (cf. Sperry and Sullivan, 1992; Sperry et al., 1994; Lipp and Nilsen, 1997; Sobrado, 1997; Sparks and Black, 2001; Cavender-Bares and Holbrook, 2001; Cordero and Nilsen, 2002; Mayr et al., 2003).

Percentage loss of conductivity is a relative measure of embolism and may not be the clearest measure of how adequately leaves are supplied with water from stems compared to the more direct measure of leaf specific conductivity (K_l) (Tyree and Ewers, 1991). On 21 January 2004, following multiple freeze–thaw events in December and January, K_l for stems of *R. ovata* was $0.45 \pm 0.16 \times 10^{-4}$ kg · s^{-1} · MPa^{-1} m^{-1}, which is low among woody angiosperms (Stratton et al., 2000; Cavender-Bares and Holbrook, 2001). At this time, stems displayed a 92% loss in conductivity. In comparison to *R. ovata*, stems for *M. laurina* around this time (3 March 2004) were less embolized (70% loss of conductivity for *M. laurina*) and

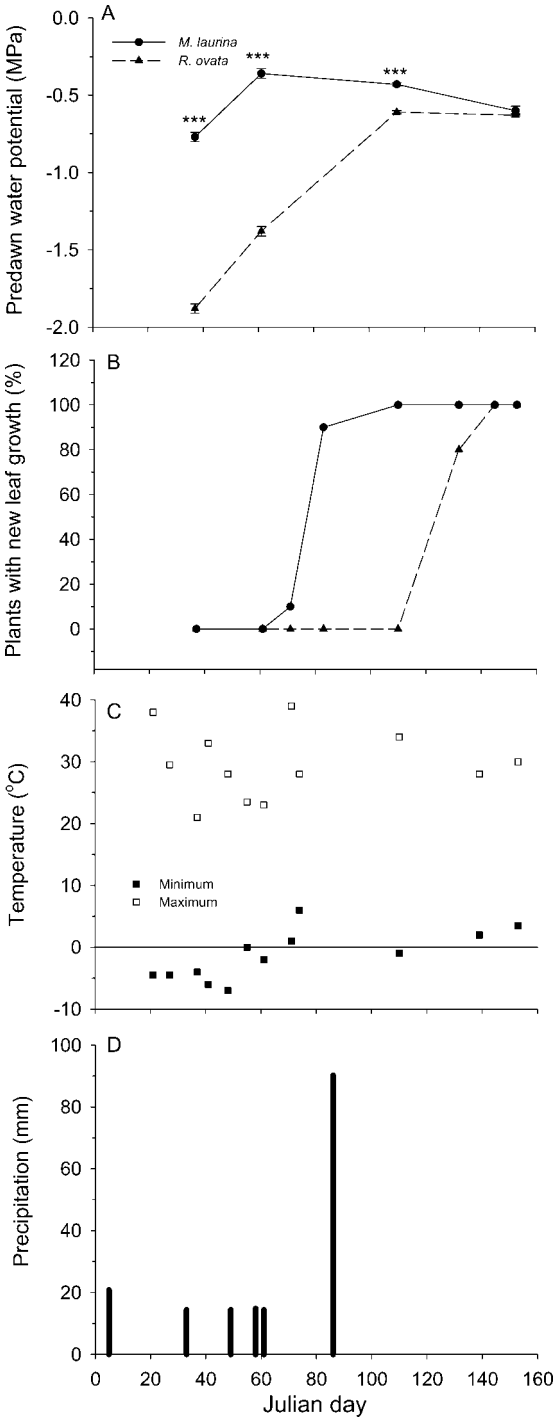

Fig. 7. Branchlet water potential at predawn (A) and percentage of plants with new leaf growth (B) for *Malosma laurina* and *Rhus ovata* in early 2004. In addition, minimum and maximum temperatures are shown for our freezing site (C). Precipitation was only collected at our warm coastal site, about 6 km south of our cold site, and is shown here as an approximation of precipitation at our cold site (D). Branchlet water potential and percentage of plants with new growth are shown as means ±1 SE ($N = 10$). Asterisks in panel A indicate significant differences between *M. laurina* and *R. ovata* (*** = $P < 0.001$).

K_l for *M. laurina* was more than five times higher at $2.37 \pm 0.42 \times 10^{-4}$ kg · s^{-1} · MPa^{-1} · m^{-1}. The high PLC and low K_l for *R. ovata* indicate that its stems were highly embolized, and water supply to its leaves was greatly reduced during winter months.

Although we focused on stems, the location of minimum conductance to water flow in plants may also be located in the roots or leaves. We did not measure the xylem conductance of roots or leaves, but we think that stem conductance is likely a chief resistance to water flow following freeze–thaw stress at our study site. This is because it is very unlikely that the roots experienced freeze–thaw stress because they are insulated by the surrounding soil. For leaves, vessel diameters are typically smaller than those in stems, which would theoretically reduce the severity of embolism following freeze–thaw stress (Davis et al., 1999; Pittermann and Sperry, 2003).

A reduction of water supply to leaves from embolism would be expected to cause a decline in Ψ_x or stomatal conductance (g_s), and we found evidence for both. The lowest published values for Ψ_x at midday for *R. ovata* are about −3.0 MPa (Poole and Miller, 1981; Vankat, 1989). Branchlet Ψ_x at midday on 6 February 2004 for *R. ovata* in our study was −3.4 MPa, which is lower than any previously published values for this species indicating that plants were under considerable water stress. In addition to low Ψ_x, *R. ovata* also exhibited low g_s. For field measurements on 6 February 2004, the maximum and minimum g_s for leaves of *R. ovata* ($g_s = 31$ and 20 mmol · m^{-2} · s^{-1}, respectively) were both about half the values measured for *M. laurina* ($g_s = 67$ and 37 mmol · m^{-2} · s^{-1}, respectively). These g_s values for *R. ovata* are extremely low compared to other woody plant species (cf. Körner, 1995), including adult chaparral species during summer drought (Davis and Mooney, 1985; Thomas and Davis, 1989).

The high degree of water stress for *R. ovata*, manifested as low Ψ_x and g_s, in February 2004 was probably not due to lack of soil moisture. The predawn and midday Ψ_x values for *M. laurina* (−0.80 and −1.69 MPa, respectively) on 6 February 2004 indicate relatively high soil moisture. January and February are typically among the wettest months in southern California, and there were multiple rainfall events in January and February 2004. Instead of lack of soil moisture, the low Ψ_x and g_s for *R. ovata* are likely due to high embolism and low K_l of stems, resulting in a reduced supply of water to its large canopy of evergreen leaves.

Cavitation caused by freezing and thawing may be a purely physical process controlled by Ψ_x and the diameter of xylem vessels. Alternatively, death of xylem parenchyma cells may contribute to losses in hydraulic conductivity of stems when they are frozen and thawed (Pockman and Sperry, 1997; Pittermann and Sperry, 2003). Davis et al. (1999) found that woody plants with hydraulic mean vessel diameters less than 35 μm at $\Psi_x = -0.5$ MPa show high resistance to cavitation caused by freeze–thaw treatments, and that plants with hydraulic mean vessel diameter greater than 35 μm are susceptible to such cavitation. Their work was confirmed by Pittermann and Sperry (2003), who found a similar critical conduit diameter for freezing susceptibility among species that have tracheids. These two studies support a physical model of freezing-induced xylem cavitation that is largely controlled by vessel or tracheid diameter. Parenchyma cells may also be involved in cavitation caused by freezing. Some plants (*Larrea tridentata* and *Ginkgo biloba*) show a negative correlation between freezing temperature and PLC (Pockman and Sperry,

1997; Pittermann and Sperry, 2003). This has been interpreted as an indication that ray parenchyma cells are progressively killed by decreasing temperatures, and this results in increasing PLC.

Our results support a physical model of cavitation caused by freezing for two reasons. First, both *M. laurina* and *R. ovata* were highly susceptible to cavitation caused by freezing, and their hydraulic mean xylem vessel diameters were greater than 35 μm. Second, we used a vital stain to assess if xylem parenchyma were killed by freezing temperatures of −10°C, and our results indicate that the parenchyma survived these freezing temperatures for both species. Because the parenchyma survived and the xylem had high PLC, we conclude that the PLC resulted primarily from the large vessel diameters and was not due to mortality of parenchyma cells. To our knowledge, these results are the first to directly examine PLC and survivorship of parenchyma for frozen stems. We do not rule out a role for parenchyma death in conductivity losses for other species, and it would be useful to assess viability of cells for *Larrea tridentata* and *Ginkgo biloba* to verify cell mortality of frozen stems.

Although the xylem parenchyma cells survived the −10°C treatment in the laboratory, such a freeze would kill shoots of *M. laurina* growing in a field environment (Fig. 2A; Langan et al., 1997). This discrepancy seems to suggest that the death of the parenchyma cells in stem-xylem following freeze–thaw stress is not immediate and that their mortality may not be directly due to freeze–thaw stress. For example, in the field, embolism caused by freeze–thaw stress may lead to the desiccation and ultimately the death of xylem parenchyma cells. Alternatively, freeze–thaw stress may injure the cells and trigger programmed cell death (apopotosis), and not until sometime later are they killed (Arora and Palta, 1988).

The leaves for *R. ovata* are clearly important for its ability to tolerate freezing temperatures in the field and for explaining its greater tolerance of freezing temperatures than *M. laurina*. Leaves for *R. ovata* adults and seedlings were not measurably damaged by freezing treatments of −10°C in the laboratory and −7.2°C in the field, respectively. In contrast, leaves for adults of *M. laurina* suffered damage at −6°C and seedlings experienced 100% mortality at −7.2°C. Freezing tolerance for *R. ovata* is better explained by the ability of its leaves to tolerate freezing temperatures and reduced water availability than the ability of its stems to resist cavitation caused by freezing. For *M. laurina*, cavitation of stems and damage to leaves both occurred at about −6°C, indicating that both are important in explaining the lack of freezing tolerance for *M. laurina*.

When extracellular ice forms it grows by withdrawing water from living cells, causing them to dehydrate (Ashworth and Pearce, 2002). Therefore, the greater tolerance of subzero temperatures for leaves of *R. ovata* may be related to their tolerance of lower Ψ_x than leaves of *M. laurina*. This appears to be the case as leaf Ψ_x for *R. ovata* declined to −5 MPa, while dark-adapted F_v/F_m remained ≥0.8 (Fig. 5C, E, H, J). By comparison, leaf Ψ_x values of −4.5 MPa for *M. laurina* lead to a decline in dark-adapted F_v/F_m values below 0.8 (Fig. 5C, E, H, J).

Another important feature of *R. ovata* leaves is that they are better able to reduce their conductance to water vapor than leaves of *M. laurina*. This was found among plants growing in the field and for experiments using excised leaves in the laboratory. For field measurements on 6 February 2004, the maximum and minimum g_s for leaves of *R. ovata* (g_s = 31 and 20 mmol · m^{-2} · s^{-1}, respectively) were about half those for co-occurring *M. laurina* (g_s = 67 and 37 mmol · m^{-2} · s^{-1}, respectively). Laboratory experiments using excised leaves to estimate minimum whole leaf conductance (stomatal and cuticular conductance; g_{leaf}) had similar results: the minimum g_{leaf} for *R. ovata* was about half that of *M. laurina*. If stems for *R. ovata* become highly embolized following a freeze–thaw event and the supply of water to the leaves is reduced, it becomes imperative that the leaves reduce their demand for water. Apparently *R. ovata* is capable of making this leaf-level adjustment. It is unclear if the difference in minimum g_{leaf} between *R. ovata* and *M. laurina* is due to differences in minimum stomatal conductance or differences in cuticular conductance.

The phenology of *R. ovata* may also enable it to grow in environments prone to winter freezing. *Rhus ovata* did not develop new leaves until 12 May 2004, which was about 2 months later than *M. laurina*. Delayed growth is an adaptation for some large-vesseled species, e.g., *Quercus* species, to avoid freezing-induced cavitation in the late spring (Sperry et al., 1994). At our field site, there are two oak species with large vessel diameters, *Q. agrifolia* and *Q. berberidifolia*, both of which leafed out about a month earlier than *R. ovata* (Pratt, personal observation). Delayed spring growth for *R. ovata* may be an adaptation to avoid late freezing events that might cause cavitation and disrupt growth in the spring and summer. However, a more likely explanation is that the high winter embolism and low predawn Ψ_x values for *R. ovata* prevented the necessary turgor pressure for cell elongation and leaf expansion.

Recovery from winter embolism for *R. ovata* probably does not involve refilling of previously embolized vessels because low Ψ_x values likely preclude refilling during the winter months. In addition, at our cold study site, native embolism for *R. ovata* has been sampled on more than 20 separate occasions and has never exhibited native PLC < 40% (S. Davis, unpublished data) suggesting that, if embolism refilling occurs, it is incomplete. We suspect that *R. ovata* largely depends on new xylem growth to supply dividing cells and developing leaves with adequate water to expand in the spring.

Bulk tissue water relations for leaves of *R. ovata* were not different from *M. laurina* and cannot explain the differential freezing tolerance of the two species. We hypothesized that greater capacitance might allow *R. ovata* to better tolerate periods of reduced water supply when K_l of stems was low compared to *M. laurina*. We also expected that *R. ovata* would have a lower osmotic potential at saturation ($\Psi_{s(sat)}$) compared to *M. laurina*, which would depress the freezing point of its cells. However, we reject both of these hypotheses because capacitance and $\Psi_{s(sat)}$ were not different between the two species (Table 1).

The seedling stage of development often has the highest mortality and determines species persistence at a site; therefore, when attempting to understand the importance of an abiotic stress on the distribution of a plant species, it is often critical to demonstrate the effect of the stress on seedling establishment. *Malosma laurina* adults do not occur in the coldest valley bottoms at our cold study site, presumably because its seedlings are killed by frost. Numerous seedlings of *R. ovata* and *M. laurina* recruited in the cold valley bottoms at our cold site after the wildfire of 1996, providing us with a rare opportunity to assess seedling performance and survivorship during freeze–thaw stress. The results of this natural ex-

periment were decisive, with seedlings of *M. laurina* showing frost damage following a −4.2°C freezing event and further damage and 100% mortality following a −7.2°C freezing event. In contrast to *M. laurina* seedlings at the cold site, co-occurring seedlings of *R. ovata* and control seedlings of *M. laurina* at a warm site showed no freezing damage and 100% of the *R. ovata* seedlings survived the subzero temperature events. These results clearly demonstrate the differential frost tolerance between seedlings of *R. ovata* and *M. laurina* and importantly show the role of freezing in limiting the distribution of *M. laurina* from colder microsites in southern California.

Freezing is often overlooked as a causal factor controlling species distributions in the Mediterranean climate region of southern California, where drought and wildfire receive more attention. In the context of climate change, freezing may be very important in southern California. Climate trends indicate that southern California has experienced 15% fewer nights with frost in the latter half of the 20th century (Christy et al., 2001) and models predict that by 2030 to 2050 winters will be 3°C warmer than they are now (Field et al., 1999). *Malosma laurina* populations in southern California often show strong recruitment from seed following wildfire or during moist conditions (Frazer and Davis, 1988; Thomas and Davis, 1989). As temperatures warm, *M. laurina* may expand its range into areas where it was previously limited by frost. Once past the second year of growth, *M. laurina* can resprout from a root crown following freezing-induced shoot death. The expansion of *M. laurina* into new microsites in the Santa Monica Mountains may be in progress.

In summary, the frost tolerance of *R. ovata* is largely explained by the ability of its leaves to tolerate freezing temperatures and desiccation and to minimize water loss from evergreen leaves when stems are highly embolized following freeze–thaw events. In contrast, *M. laurina* has none of these adaptations and suffers severe frost damage at the seedling and adult stages of development. *Rhus ovata* may be unique among evergreen woody plants in tolerating high levels of cavitation caused by freezing instead of avoiding cavitation, which based upon the literature, appears to be a more common strategy.

LITERATURE CITED

ARORA, R., AND J. P. PALTA. 1988. *In vivo* perturbation of membrane-associated calcium by freeze–thaw stress in onion cells. *Plant Physiology* 87: 622–628.

ASHWORTH, E. N., AND R. S. PEARCE. 2002. Extracellular freezing in leaves of freezing-sensitive species. *Planta* 214: 798–805.

BOORSE, G. C., T. L. BOSMA, A.-C. MEYER, F. W. EWERS, AND S. D. DAVIS. 1998a. Comparative methods of estimating freezing temperatures and freezing injury in leaves of chaparral shrubs. *International Journal of Plant Sciences* 159: 513–521.

BOORSE, G. C., F. W. EWERS, AND S. D. DAVIS. 1998b. Differential response of chaparral shrubs to below freezing temperatures. *American Journal of Botany* 85: 1224–1230.

CAMPBELL, G. S., AND J. M. NORMAN. 1998. An introduction to environmental biophysics. Springer-Verlag, New York, New York, USA.

CARLQUIST, S. W., AND D. A. HOEKMAN. 1985. Ecological wood anatomy of the woody southern California flora. *IAWA Bulletin* 6: 319–347.

CAVENDER-BARES, J., AND N. M. HOLBROOK. 2001. Hydraulic properties and freezing-induced cavitation in sympatric evergreen and deciduous oaks with contrasting habits. *Plant, Cell and Environment* 24: 1243–1256.

CHRISTY, J. R., R. A. CLARKE, G. V. GRUZA, J. JOUZEL, M. E. MANN, J. OERLEMANS, M. J. SALINGER, AND S. W. WANG. 2001. *In* J. T. Houghton, Y. Ding, D. J. Griggs, M. Noguer, P. J. van der Linden, X. Dai, K. Maskell, and C. A. Johnson [eds.], Climate change 2001: The scientific basis, 164–181. Cambridge University Press, New York, New York, USA.

CORDERO, R. A., AND E. T. NILSEN. 2002. Effects of summer drought and winter freezing on stem hydraulic conductivity of *Rhododendron* species from contrasting climates. *Tree Physiology* 22: 919–928.

DAVIS, S. D., F. W. EWERS, J. S. SPERRY, K. A. PORTWOOD, M. C. CROCKER, AND G. C. ADAMS. 2002. Shoot dieback during prolonged drought in *Ceanothus* (Rhamnaceae) chaparral of California: a possible case of hydraulic failure. *American Journal of Botany* 89: 820–828.

DAVIS, S. D., AND H. A. MOONEY. 1985. Comparative water relations of adjacent California shrub and grassland communities. *Oecologia* 66: 522–529.

DAVIS, S. D., J. S. SPERRY, AND U. HACKE. 1999. The relationship between xylem conduit diameter and cavitation caused by freeze–thaw events. *American Journal of Botany* 86: 1367–1372.

DESOUZA, J., P. SILKA, AND S. D. DAVIS. 1986. Comparative physiology of burned and unburned *Rhus laurina* after chaparral wildfire. *Oecologia* 71: 63–68.

EWERS, F. W., M. C. LAWSON, T. J. BOWEN, AND S. D. DAVIS. 2003. Freeze/thaw stress of *Ceanothus* of southern California chaparral. *Oecologia* 136: 213–219.

FIELD, C. B., G. C. DAILY, F. W. DAVIS, S. GAINES, P. A. MATSON, J. MELACK, AND N. L. MILLER. 1999. Confronting climate change in California: ecological impacts on the golden state, 1–63. Union of Concerned Scientists, Cambridge, Massachusetts, USA, and Ecological Society of America, Washington D.C., USA.

FRAZER, J. M., AND S. D. DAVIS. 1988. Differential survival of chaparral seedlings during the first summer drought after wildfire. *Oecologia* 76: 215–221.

HICKMAN, J. C. 1993. The Jepson manual. University of California Press, Berkeley, California, USA.

KERSTIENS, G. 1996. Cuticular water permeability and its physiological significance. *Journal of Experimental Botany* 47: 1818–1832.

KOIDE, R. T., R. H. ROBICHAUX, S. R. MORSE, AND C. M. SMITH. 1989. Plant water status, hydraulic resistance and capacitance. *In* R. W. Pearcy, J. Ehleringer, H. A. Mooney, and P. W. Rundel [eds.], Plant physiological ecology: field methods and instrumentation, 161–183. Champman and Hall, London, UK.

KÖRNER, C. 1995. Leaf diffusive conductances in the major vegetation types of the globe. *In* E.-D. Schultze and M. M. Caldwell [eds.], Ecophysiology of photosynthesis, 463–490. Springer-Verlag, New York, New York, USA.

KUBISKE, M. E., AND M. D. ABRAMS. 1991. Rehydration effects on pressure–volume relationships in four temperate woody species: variability with site, time of season and drought conditions. *Oecologia* 85: 537–542.

LANGAN, S. J., F. W. EWERS, AND S. D. DAVIS. 1997. Differential susceptibility to xylem embolism caused by freezing and water stress in two species of chaparral shrubs. *Plant, Cell and Environment* 20: 425–437.

LARCHER, W. 1995. Physiological plant ecology. Springer-Verlag, New York, New York, USA.

LIPP, C. C., AND E. T. NILSEN. 1997. The impact of subcanopy light environment on the hydraulic vulnerability of *Rhododendron maximum* to freeze–thaw cycles and drought. *Plant, Cell and Environment* 20: 1264–1272.

LOIK, M. E., AND S. P. REDAR. 2003. Microclimate, freezing tolerance, and cold acclimation along an elevation gradient for seedlings of the Great Basin shrub, *Artemesia tridentata*. *Journal of Arid Environments* 54: 769–782.

MARINEZ-VILALTA, J., AND W. T. POCKMAN. 2002. The vulnerability to freezing-induced xylem cavitation of *Larrea tridentata* (Zygophyllaceae) in the Chihuahuan desert. *American Journal of Botany* 89: 1916–1924.

MAYR, S., F. SCHWIENBACHER, AND H. BAUER. 2003. Winter at the alpine timberline. Why does embolism occur in Norway spruce but not in stone pine? *Plant Physiology* 131: 780–792.

MILLER, P. C., AND D. K. POOLE. 1979. Patterns of water use in shrubs in southern California. *Forest Science* 25: 84–98.

PERCIVAL, G. C., AND A. HENDERSON. 2003. An assessment of the freezing tolerance of urban trees using chlorophyll fluorescence. *Journal of Horticultural Science and Biotechnology* 78: 254–260.

PITTERMANN, J., AND J. SPERRY. 2003. Tracheid diameter is the key trait determining the extent of freezing-induced embolism in conifers. *Tree Physiology* 23: 907–914.

POCKMAN, W. T., AND J. S. SPERRY. 1997. Freezing-induced xylem cavitation and the northern limit of *Larrea tridentata*. *Oecologia* 109: 19–27.

POOLE, D. K., AND P. C. MILLER. 1981. The distribution of plant water stress and vegetation characteristics in southern California chaparral. *The American Midland Naturalist* 105: 32–43.

ROTMAN, B., AND B. W. PAPERMASTER. 1966. Membrane properties of living mammalian cells as studied by enzymatic hydrolysis of fluorogenic esters. *Proceedings of the National Academy of Sciences, USA* 55: 134–141.

SAKAI, A., AND W. LARCHER. 1987. Frost survival in plants: responses and adaptation to freezing stress. Springer-Verlag, New York, New York, USA.

SOBRADO, M. A. 1997. Embolism vulnerability in drought-deciduous and evergreen species of a tropical dry forest. *Acta Oecologia* 18: 383–391.

SPARKS, J. P., AND R. A. BLACK. 2001. Winter hydraulic conductivity and xylem cavitation in coniferous trees from upper and lower treeline. *Arctic Antarctic Alpine Research* 32: 397–403.

SPERRY, J. S., J. R. DONNELLY, AND M. T. TYREE. 1988. A method for measuring hydraulic conductivity and embolism in xylem. *Plant, Cell and Environment* 11: 35–40.

SPERRY, J. S., K. L. NICHOLS, J. E. M. SULLIVAN, AND S. E. EASTLACK. 1994. Xylem embolism in ring-porous, diffuse-porous, and coniferous trees of northern Utah and interior Alaska. *Ecology* 75: 1736–1752.

SPERRY, J. S., AND J. E. M. SULLIVAN. 1992. Xylem embolism in response to freeze–thaw cycled and water stress in ring-porous, diffuse-porous, and conifer species. *Plant Physiology* 100: 605–613.

STILLER, V., AND J. S. SPERRY. 2001. Canny's compensating pressure theory fails a test. *American Journal of Botany* 86: 1082–1086.

STRATTON, L., G. GOLDSTEIN, AND F. C. MEINZER. 2000. Stem water storage capacity and efficiency of water transport: their functional significance in a Hawaiian dry forest. *Plant, Cell and Environment* 23: 99–106.

THOMAS, C. M., AND S. D. DAVIS. 1989. Recovery patterns of three chaparral shrub species after wildfire. *Oecologia* 80: 309–320.

TYREE, M. T., AND F. W. EWERS. 1991. The hydraulic architecture of trees and other woody plants. *New Phytologist* 119: 345–360.

TYREE, M. T., AND H. T. HAMMEL. 1972. The measurement of the turgor pressure and water relations of plants by the pressure bomb technique. *Journal of Experimental Botany* 23: 267–282.

VANKAT, J. L. 1989. Water stress in chaparral shrubs in summer-rain versus summer-drought climates—-whither the Mediterranean-type climate paradigm? *In* S. Keeley [ed.], The California chaparral: paradigms reexamined, 117–124. Natural History Museum of Los Angeles County, Los Angeles, California, USA.

WINDHOLM, J. M. 1972. The use of fluorescein diacetate and phenosafranine for determining viability of cultured plant cells. *Stain Technology* 47: 189–194.

YANG, S., AND M. T. TYREE. 1992. A theoretical model of hydraulic conductivity recovery from embolism with comparison to experimental data on *Acer saccharum*. *Plant, Cell and Environment* 15: 633–643.