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# Correlation Between Freezing Sites and Xylem Vessel Diameter for Three Chaparral Species of the Santa Monica Mountains

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## Abstract

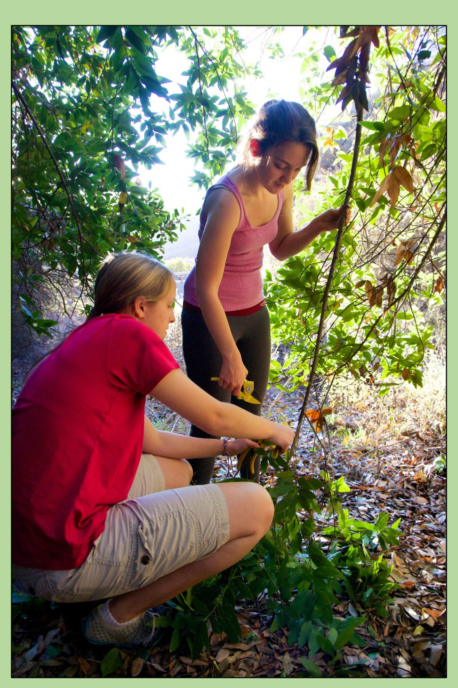
Coastal exposures of the Santa Monica Mountains rarely experience freezing temperatures (0 °C) because of the ameliorating effects of the Pacific Ocean and seawater's specific heat capacity. In contrast, inland sites of the Santa Monica Mountains frequently experience winter temperatures below -10 °C. This temperature gradient, from coast to inland, may be a major determinate of species distribution patterns. To investigate possible mechanisms by which freezing impacts chaparral distribution patterns, we examined xylem vessel diameter and vessel length of three chaparral species growing at inland freezing sites versus coastal nonfreezing sites (*Malosma laurina, Umbellularia californica,* and *Ceanothus megacarpus*). It has been established that vessel size influences freezing-induced embolism and the blockage of xylem water transport from soil to leaves. However, it is not known if this "size effect" is primarily due to vessel diameter, vessel length, or both. We initially hypothesized that matched species-pairs at non-freezing sites would have both longer and wider vessels than at freezing sites. We determined maximum vessel length by injecting air into stems at decreasing segment lengths and mean vessel diameters by using an ocular micrometer in conjunction with a light microscope. Sample sizes were six for each species pairs. For all three species, mean vessel diameters were narrower at freezing than non-freezing sites ($P < 0.05$) ranging between 13 µm mean differences for *C. megacarpus* to 20.4 µm mean differences for *U. californica*. In contrast, we found no significant difference in vessel lengths for any of the species-pairs ($P > 0.05$). We conclude that reduction in vessel diameter is more significant than reduction in vessel length for protection from freezing-induced embolism of stem xylem. Furthermore, limits in the genetic plasticity of some species to reduce vessel diameter may preclude their survival at freezing sites.

## Introduction

Observations in the Santa Monica Mountains located in Malibu, California have shown that there are physical and physiological variations in the plants based on environment's varying temperatures. Lower temperatures have been recorded at the bases of the hills due to heat being less dense than cold air. Significant differences in outside temperatures may be affecting plants. Plants on the lower ends of the hills are shown to have more red leaves because the freezing conditions cause the water in the xylem of the plants to freeze causing gas bubbles to form, ultimately resulting in embolism, the blockage of water flow. Speciation across the different temperature gradients vary due to adaptations to the extreme conditions. Plants with larger xylems experience higher mortality rates due to the coagulation of large gas bubbles that form in large-diameter xylem conduits that will damage the plant (Davis et al. 2007). In other species, freezing has been shown to increase vulnerability to cavitation with increasing diameter length, but showed no loss of conductivity (Davis et. al 1999). Cavitation is the formation of a gas in the xylems of plants. Cavitation can be detrimental to plants that freeze and thaw, because gas bubbles may cause embolism and ultimately death to the plant. Freezing-induced embolism correlates with the diameter of the xylem. Xylem diameter determines the sizes of the gas bubbles, and large bubbles in large xylem take longer to thaw and are more likely to expand than are those of smaller xylem diameters (Langan et. al 1997). Longer thawing durations and larger expansions will result in higher plant mortality rates. Freezing seedlings could affect the long-term plant distribution patterns in the Santa Monica Mountains. In addition, hydrated freezing conditions will cause the plants' embolism to occur at a more extreme levels (Pratt et. al 2007). We hypothesize that in freezing zones, *M. laurina*, *U. californica*, and *C. megacarpus* will have shorter xylem diameters as opposed to those in non-freezing zones. In addition, we predict that these changes will result in less conductivity in both species.

## Description of Study Site

Our study sites consisted of locations in the Santa Monica Mountains, including Tapia Park, the Malibu Forestry Unit of Los Angeles County, Solstice Canyon, and Pepperdine University's Malibu campus. From temperature data collected over the past few years, Tapia Park reaches freezing temperatures as low as -12°C, while Pepperdine University and Solstice Canyon do not (Davis et al.). We collected freezing *M. laurina* from the Malibu Forestry Unit of Los Angeles, freezing *U. californica* samples from Tapia Park, non-freezing *M. laurina* samples from Pepperdine University, and non-freezing *U. californica* samples from Solstice Canyon.

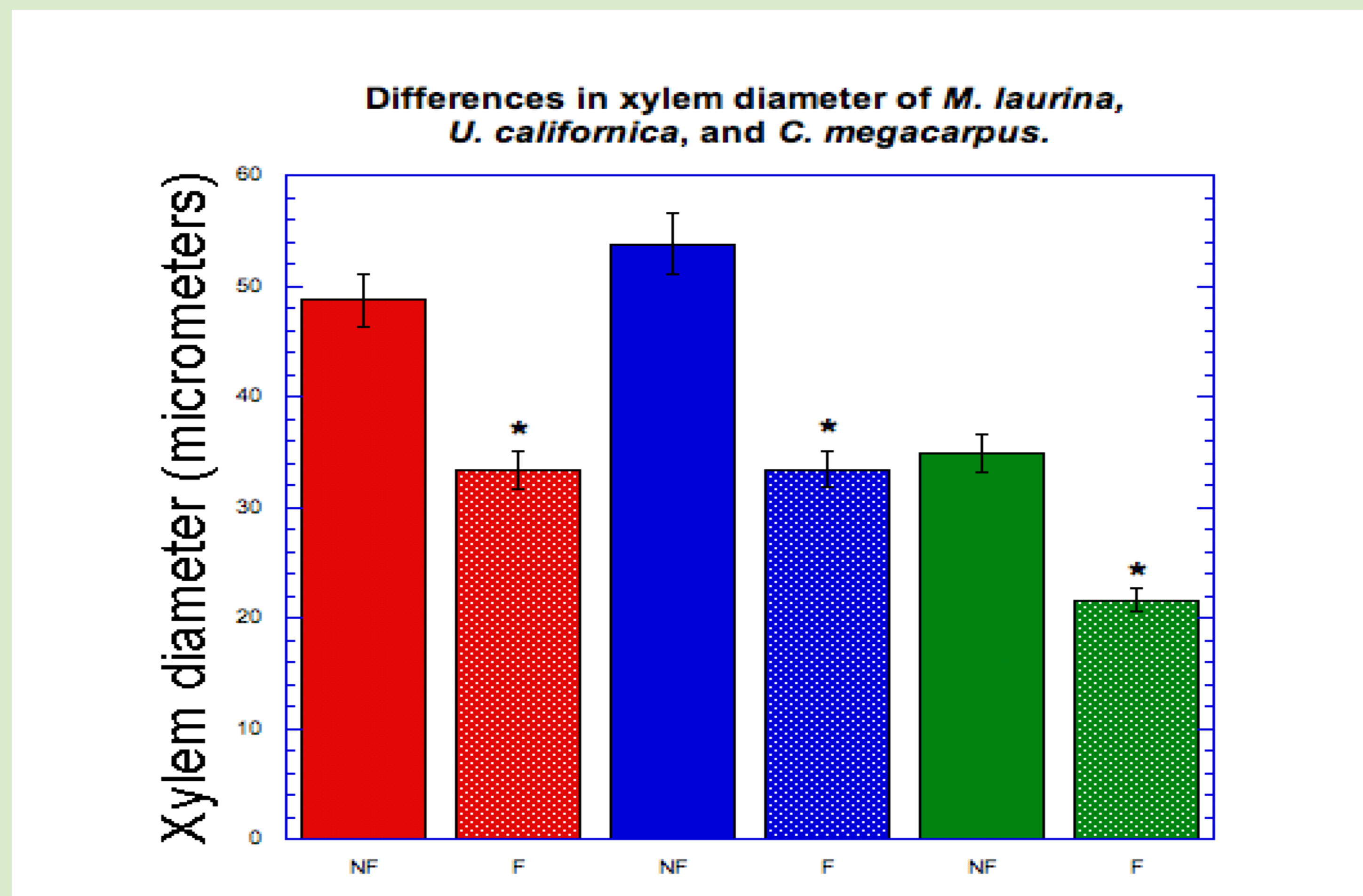


Left: Example of freezing *U. californica*, found in Tapia Park, Malibu, California.

## Methods

Using a random number generator, we selected six plants from each species in both non-freezing and freezing areas to collect samples from. We measured the diameter of branches using calipers and cut off three branch sections from each plant measuring between 7 and 9 millimeters. We stored the branch sections on ice until taken back to the lab. Our control groups were the non-freezing plants of all three species and our experimental groups were the freezing plants of all three species. Once back in the lab, we randomly selected one of the three branches taken from each individual plant and used a razor blade to cut thin cross-sections. We prepared microscope slides by mounting the cross-sections onto slides in water. In a microscope with a built-in ruler under 400x magnification, we measured the greatest diameter of each xylem in an area between two neighboring vascular rays. The greatest diameter was used because a xylem is rarely a perfect circle, so we set this standard to keep measurements more consistent. We used an unpaired student T-test to show that our results are statistically significant.

## Results

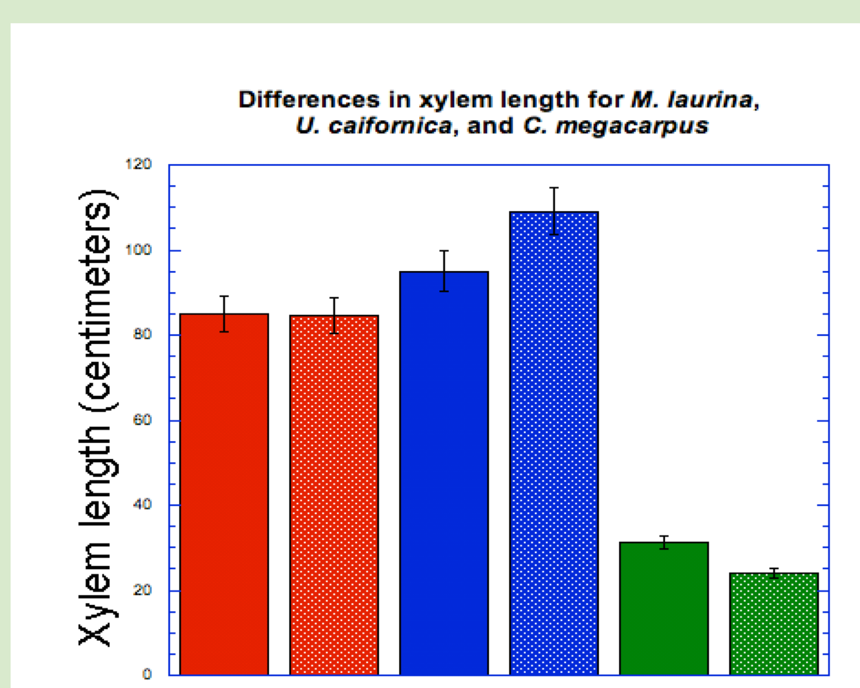



*M. laurina* *U. californica* *C. megacarpus*

**Figure 1. Xylem diameters at non-freezing (NF) and freezing (F) sites for *M. laurina*, *U. californica*, and *C. megacarpus*.** An asterisk denotes significant difference between non-freezing and freezing sites at $P < 0.05$ by Student's t-test. Solid bars represent plants from freezing areas, patterned bars represent plants from non-freezing areas. Bars on symbols represent ± 1 SE, n= 6.

| | *M. laurina* | | *U. californica* | | *C. megacarpus* | |
|---|---|---|---|---|---|---|
| | NF | F | NF | F | NF | F |
| Predicted Freeze Cavitation [Davis et al. 1999] | 99% | 35% | 100% | 35% | 50% | 2% |

**Figure 2. Predicted cavitation in percent based on mean vessel diameters measured in this report and vulnerability curves to freezing induced cavitation reported by Davis et al. 1999, also see Langan et al. 1997.** Non-freezing sites were located near the Pacific Ocean, which prevents nighttime temperatures from dipping below 0°C, whereas freezing sites are inland from the ocean and experience freezing temperatures as low as -12°C.




*M. laurina* *U. californica* *C. megacarpus*

**Figure 3. Xylem lengths at non-freezing (NF) and freezing (F) sites for *M. laurina*, *U. californica*, and *C. megacarpus*.** Solid bars represent plants from freezing areas, patterned bars represent plants from non-freezing areas. Bars on symbols represent ± 1 SE, n= 6. Xylem lengths between non-freezing and freezing sites were not significantly different by Student's t-test at $P > 0.05$.

## Discussion

Our original hypothesis stated that *Malosma laurina, Umbellularia californica,* and *Ceanothus megacarpus* in freezing zones will have smaller xylem diameters compared to those in non-freezing zones. Our results supported our hypothesis, and showed that harsh freezing conditions do negatively affect these plants' xylem diameters. Previously conducted research in this field has also shown similar results. Estimates show that the relationship between mean conduit diameters (nanometers) plotted against cavitation of frozen and non-frozen plant stems creates a curve that relates mean conduit diameter to cavitation (Davis et al.). When comparing our mean values for freezing and non-freezing *M. laurina*, *U. californica,* and *C. megacarpus* to the published curve, we notice that the non-freezing plants have a higher mean value than the freezing subjects, and higher percent cavitation. Non-freezing *M. laurina* has approximately 99% cavitation, whereas *U. californica* has 100% cavitation and *C. megacarpus* has 50% cavitation. Freezing *M. laurina* has 35% cavitation, whereas *U. californica* has about 35% cavitation and *C. megacarpus* has 2% cavitation. Freezing *M. laurina* will see around a 64% loss in conductivity by freezing, *U. californica* will experience a 65% loss, and *C. megacarpus* will experience a 48% loss. Their results also showed that non-freezing *M. laurina* will experience 95% productivity, and non-freezing *U. californica* will not experience any changes at all in non-freezing areas (Davis et al.). This publication reaffirms our results because non-freezing stems would have a higher chance of embolism due to their larger xylem diameters, whereas freezing stems would have a lower chance of embolism due to smaller diameters. Freezing stems have a lower percent cavitation because they have smaller diameters that are not as likely to have embolism than stems with larger xylem diameters. Therefore, from these results we can conclude that harsh conditions hinder the chaparral plants' abilities to transport the maximum amount of water throughout their vascular systems. Ultimately, the vessel length was determined to not be significant, while the vessel diameter did yield significant results.

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