

2013

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Nicole A.P.M.K.O.M. Nakamatsu
Pepperdine University

Theadora V. Ordog
Pepperdine University

Kaitlyn E. Sauer
Pepperdine University

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Recommended Citation

Nakamatsu, Nicole A.P.M.K.O.M.; Ordog, Theadora V.; and Sauer, Kaitlyn E., "The Effect of Freezing Conditions on Xylem Diameter of *Malosma laurina* and *Umbellularia californica* in the Santa Monica Mountains" (2013). Pepperdine University, *Featured Research*. Paper 130.
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The Effect of Freezing Conditions on Xylem Diameter of *Malosma laurina* and *Umbellularia californica* in the Santa Monica Mountains

Nicole A. P. M. K. O. M. Nakamatsu, Theadora V. Ordog, and Kaitlyn E. Sauer
Mentor: Dr. Stephen D. Davis



Pepperdine University, 24255 Pacific Coast Highway, Malibu, CA 90263

Abstract

Temperature variations in the Santa Monica Mountains are drastic at times, which may be affecting the local plants. To determine the repercussions of these variations in temperature, we measured the xylem diameters of freezing and non-freezing *Malosma laurina* and *Umbellularia californica*. *U. californica* was chosen because it has a large xylem diameter, yet is known to survive freezing conditions. We thought that this plant could provide the most significant results to prove or disprove our hypothesis. *M. laurina* was chosen because it is abundant in the Santa Monica Mountains and has different physical dimensions than *U. californica*. Smaller xylem diameters may affect water transport, possibly resulting in physically smaller plant sizes. Natural selection may be acting on these species in the Santa Monica Mountains, eliminating plants with larger xylem diameters in freezing areas. We hypothesize that *M. laurina* and *U. californica* in freezing zones will have smaller xylem diameters compared to those in non-freezing zones. We collected samples of *M. laurina* and *U. californica* from freezing and non-freezing sites located in Tapia Park and Solstice park in the Santa Monica Mountains and on Pepperdine University's Malibu Campus. We then prepared slides of the plants' stems and measured the diameters of the xylems. Using Kaleidagraph, we statistically analyzed our data and found the average values for each plant; freezing *M. laurina* had an average xylem diameter of 33.3 μm , non-freezing *M. laurina* had an average xylem diameter of 48.7 μm , freezing *U. californica* had an average xylem diameter of 33.4 μm , and non-freezing *U. californica* had an average xylem diameter of 53.8 μm . These results indicate significance as revealed by a p value <0.05.

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 surrounding 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 have effects on the species. 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 (Helms 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 (Hacke 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 death to the plant. Freezing-induced embolism correlates with the diameter of, and therefore the volume within, 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* and *U. californica* 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. To test this, we collected samples of both plants from both freezing and non-freezing zones. We then took cross-sections of each branch and use a microscope with a built-in ruler to measure the diameter of the xylem in the lab.

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 park, and Pepperdine University's Malibu campus. From temperature data collected over the past years, we know that Tapia park reaches freezing temperatures, while Pepperdine University and Solstice park do not. We collected freezing *M. laurina* from the Malibu Forestry Unit of Los Angeles, freezing *U. californica* samples from Tapia Park, nonfreezing *M. laurina* samples from Pepperdine University, and nonfreezing *U. californica* samples from Solstice park.



Example of one of the species studied, freezing *U. californica*, found in Tapia park, Malibu, California.

Methods

Using a random number generator, we selected six plants from each group to collect samples from. We measured the diameter of branches using calipers and cut off 3 branch sections from each plant measuring between 7 and 9 millimeters. This process was completed in both freezing and nonfreezing areas. We stored the branch sections on ice until taken back to the lab. Our control groups were the nonfreezing plants of both species and our experimental groups were the freezing plants of both 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 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

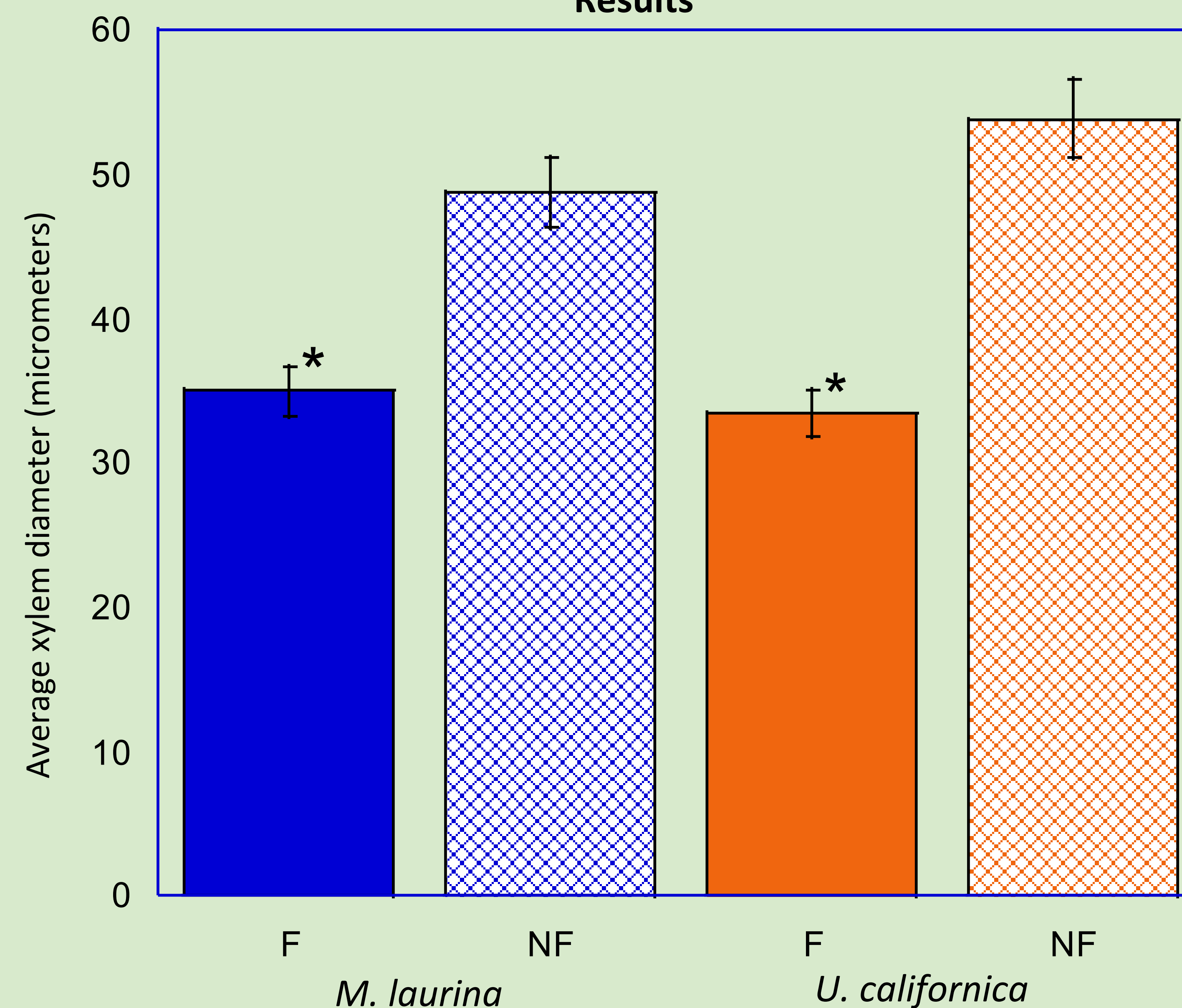
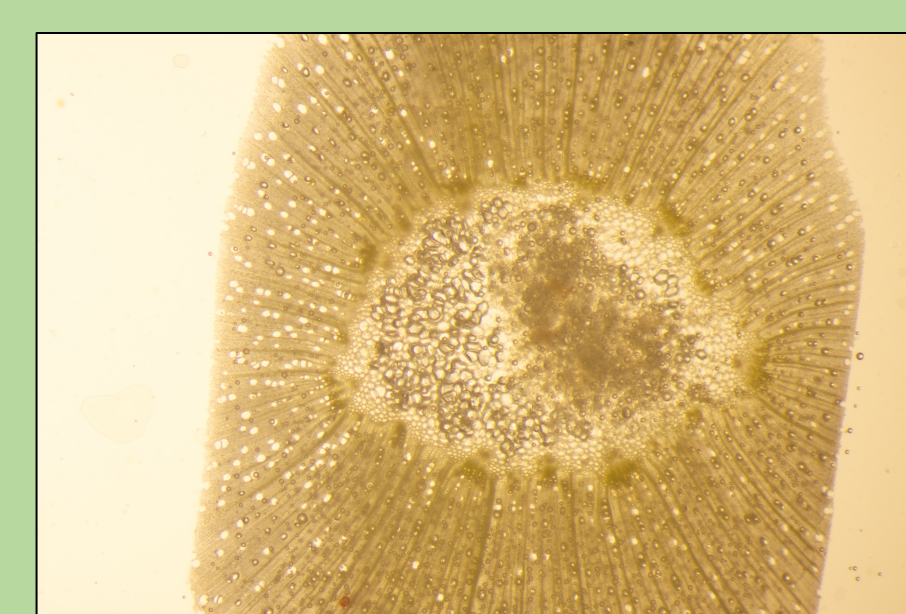


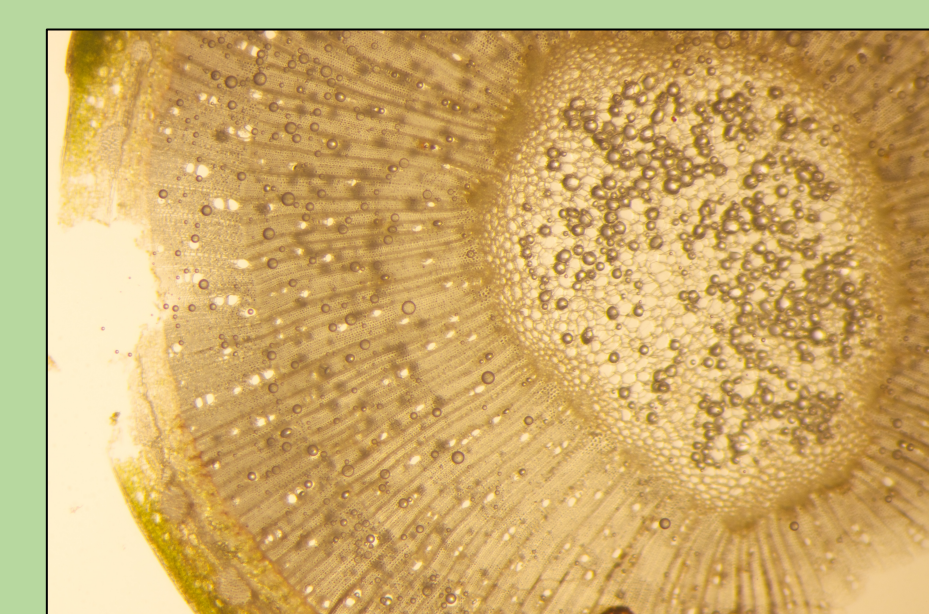
Figure 1. Average xylem diameters in micrometers of freezing (F) and non-freezing (NF) *Malosma laurina* and *Umbellularia californica*. Blue bars represent *M. laurina*, whereas orange bars represent *U. californica*. Solid bars represent freezing subjects, whereas checkered bars represent non freezing subjects. *denotes T probability is less than 0.05, indicating significance in results. Bars represent ± 1 standard error. n= 6.

	Freezing <i>M. laurina</i>	Non-freezing <i>M. laurina</i>	Freezing <i>U. californica</i>	Non-freezing <i>U. californica</i>
Hacke curve (% cavitation)	80%	98%	85%	100%
Pittermann curve (% cavitation)	83%	90%	90%	100%

Figure 2. % cavitation of Freezing *M. laurina*, non-freezing *M. laurina*, freezing *U. californica*, and non-freezing *U. californica* according to the Hacke and Pittermann curves. Non-freezing subjects have a higher % cavitation, and thus a higher probability of embolism due to larger xylem diameters, whereas freezing subjects have a lower % cavitation, and this lower probability of embolism due to smaller xylem diameters.



Cross-section of Freezing *M. laurina* from the Malibu Forestry Unit of Los Angeles.



Cross-section of Freezing *U. californica* from Tapia Park.

Discussion

Our original hypothesis stated that *Malosma laurina* and *Umbellularia californica* 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. According to an article by U. G. Hacke, estimates show that the relationship between mean conduit diameters (micrometers) was plotted against cavitation of frozen and non-frozen plant stems, in order to create a curve that relates mean conduit diameter to cavitation (Hacke). When comparing our mean values for freezing and non-freezing *M. laurina* and *U. californica* to the published curve, we notice that non-freezing *M. laurina* and non-freezing *U. californica* has a higher mean value than the freezing subjects, and a nearly 100% cavitation. Non-freezing *M. laurina* has approximately 98% cavitation, whereas non-freezing *U. californica* has 100% cavitation. Freezing *M. laurina* has 80% cavitation, whereas freezing *U. californica* has about 85% cavitation. Freezing *M. laurina* will see around a 20% loss in conductivity by freezing, and *U. californica* will also experience a 20% loss as well. 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 (Hacke). This publication reaffirms our results because non-freezing stems would have a higher chance of embolism because of their larger xylem diameters, whereas freezing stems would have a lower chance of embolism due to smaller diameters. Freezing stems have a lower % cavitation because they, in essence, have smaller diameters that are not as likely to have embolism than stems with larger xylem diameters. While we did not test the latter portion of Hacke's discoveries, their results relating to xylem size coincide with ours, and further show that our data correlates with other professionals' results. 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. Another publication (Pittermann) exhibits a similar curve as the Hacke publication, where loss of conductivity (cavitation) is plotted against mean tracheid diameter (micrometers) in conifers. In comparing the data presented in the publication with our results, we notice that non-freezing *M. laurina* would have 90% cavitation, freezing *M. laurina* would have 83% cavitation, non-freezing *U. californica* would have 100% cavitation, and freezing *U. californica* would have 90% cavitation. According to our obtained results and the results from the Pittermann publication, we could perhaps relate our findings to conifers as well.

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Acknowledgements

This research was funded by a grant from the W. M. Keck Foundation, Research Experience for Undergraduates, #DBI-106272, and the Natural Science Division of Pepperdine University. We'd like to thank Dr. Stephen Davis for his continued support and guidance in our research. We'd also like to thank Steven Fleming for his support in our research.

