Final Technical Report for Award No. DE-FG02-07ER64393
Project Title: HYDRAULIC MECHANISMS OF SURVIVAL AND MORTALITY DURING DROUGHT IN PINON JUNIPER WOODLANDS OF SOUTHWESTERN USA
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A collaboration (via separate award) with Nate McDowell, Los Alamos National Lab

Executive Summary

This study was designed to understand the mechanisms that are responsible for the differential mortality of pinon (*Pinus edulis*) and juniper (*Juniperus monosperma*) during drought in woodlands of the southwestern United States. During periodic severe drought in this region, pinon mortality is often more extensive than in co-occurring juniper yet the mechanism of this differential response is poorly understood. We approached this goal by manipulating available water on experimental plots at the Sevilleta National Wildlife Refuge in central New Mexico. We altered water availability by imposing drought by deploying plastic troughs to intercept ~45% of ambient rainfall or by increasing growing season water availability through the addition of six 19 mm irrigation events. In addition to treatment plots, we established untreated control plots with no experimental infrastructure and rainout control plots with the same trough structures installed to measure the effects of the structures without altering the amount of ambient precipitation reaching the soil.

We observed rapid pinon mortality on the two hillslope drought plots after one year of treatment. Bark beetle outbreaks in the area around the site complicated the interpretation of this drought-induced mortality and led us to establish (2010) an additional control and drought plot with insecticide application to control bark beetles. A third drought plot with deeper soils and less slope did not exhibit mortality until the fourth year of the study. Although the drought treatment impaired juniper function, mortality was essentially non-existent among junipers. The insecticide plots showed similarly severe signs of drought stress but no mortality was observed after more than 5 years of drought/insecticide treatment. This result suggests that pinon drought tolerance is well developed but that the presence of bark beetles can accelerate the impacts of drought compared to trees that do not experience bark beetle activity. Our data suggest that both water transport limitations and the effects of carbon limitations during extended periods without photosynthetic carbon gain are contributing to pinon mortality. These results make an important contribution to the rapidly growing literature on the mechanisms of drought induced mortality and expand our understanding of the mortality mechanisms in the very widespread pinon-juniper woodland biome.

Comparison of Project Goals and Accomplishments with Actual Progress.

Actual progress was well-matched with the project goals stated in our original proposal. We sought to implement a long-term precipitation manipulation experiment to test hypotheses related to the mechanism of mortality in woody species. We established the initial treatments in 2007, observed pinon mortality in hillslope drought plots in 2008 and on flat deep soils after four years. The high activity of bark beetles in the original experiment led us to add an insecticide treatment that provided added information about the drought tolerance of pinon in the absence of bark beetles. In these hillslope plots, pinon survived more than five years of drought. The long-term data collected for this project have been in high demand from the modeling community as evidenced by the five papers (see below) that report on modeling studies using our site as a test bed.

Summary of Project Activities.

The goal of this project is to use rainfall manipulation of an intact pinon-juniper woodland in central New Mexico to understand the mechanisms that control the response of these species to extremes of rainfall.

A brief timeline of the project:

2005-2006: Proposal, site selection and initial site setup
Summer 2006 - Summer 2007: Site construction
August 2007: Treatment begins
August 2008: Pinon mortality in drought plots on hillslopes with shallow rocky soils.
August 2009 to Present: Progressive juniper chlorosis, dieback and limited mortality in drought plots with shallow rocky soils. Pinon and juniper on deeper soils with low slope exhibit measurable effects of drought treatment but do not exhibit signs of mortality.
December 2010: Treatment begins on bark beetle control plot (40 x 40 m drought and control plots)
December 2011: Continued juniper mortality on hillslope blocks, no mortality on the block with deeper soil and dieback of small juniper on the new spray drought plot.
Fall 2012: One additional juniper died on hillslope plot, conducted "reference watering" experiment to document response of surviving target trees in control, irrigation and drought plots to very high soil moisture to reveal cumulative changes associated with treatment.
Fall 2013: Irrigation treatments are concluded.
Fall 2016: Drought structures (except for pesticide control experiment) are removed.

We manipulated rainfall on 40 x 40 m plots and measure target trees of pinon and juniper in the center of each plot. We reduced precipitation using rainfall diversion structures consisting of permanently placed troughs of plastic, roughly 1 m above the soil surface, that carry water away roughly 50% of rainfall falling on the treated area to collection gutters at the plot. Our water addition treatments applied high-quality water trucked to the site from Albuquerque to reduce water stress and simulate increased precipitation by adding six large storms (~22 mm equivalent) from early spring through fall, with water applied from 16 aerial sprinklers distributed across each plot to simulate rainfall. In addition to three untreated control plots, our design also included three rainout control plots that allowed us to distinguish the effects of the rainfall treatment from the effects of modified environment caused by the presence of plastic troughs over the plot surface. All treatments were implemented in August 2007. These methods are described in detail in Pangle et al. (2012) including the relatively minor effects on air and soil temperature revealed by comparison of cover-control (plastic but no rain diversion) with untreated control plots.

After 1 year of rainout treatment (Aug 2008), drought pinon on our hillslope plots were attacked by bark beetles (*Ips confusus*), infected with bluestain fungus (*Ophiostoma* spp.), and subsequently suffered 65% mortality (with complete pinon mortality on these plots by Aug 2009); no mortality was observed on the control plots. Application of a hydraulic model showed that hydraulic limitation of gas exchange is consistent with carbon limitation as an ultimate cause of drought-related mortality (in this case facilitated by beetle/fungal attack), and possibly as a proximal cause. Our measurements of hydraulic conductance do not indicate that mortality is simply the result of hydraulic failure (Plaut et al. 2012).

The 2008 mortality eliminated pinon from the drought treatment of our original intensively measured block (our design features 3 blocks but initially only one was intensively instrumented because of the effort/cost). To provide additional perspective on the mechanisms of mortality, we instrumented target trees in all plots of the remaining two blocks, both of which might be expected to respond more slowly because of deeper soils (1 block) or less extreme exposure (1 block) than the block where mortality occurred rapidly. We also added heat ratio probes to better document low flow conditions that may distinguish differences among treatments.

In 2008, our ability to infer mechanisms of mortality was complicated by high levels of bark beetle activity in the area (among the highest detected by USFS during bark beetle survey flights in the region). Bark beetles build galleries under the bark of infected trees, potentially influencing phloem function of these trees, and serve as a vector for the fungus *Ophiostoma* spp. which blocks the xylem of infected trees. In 2010 (with supplemental funding from DOE to LANL and a subcontract to UNM) we established an additional drought plot using trees sprayed to control bark beetles for one year prior to the start of the drought treatment (drought structure completed December 6 2010). Our sprayed drought plot will provide information about how trees in a similar setting (rocky slopes with shallow soils respond to drought when bark beetle activity is controlled). This plot and adjacent control trees are being used for additional measurements like those in our existing experiment to test the effect of drought on pinon in the absence of bark beetle activity.

Allometry of pinon and juniper: Initial allometric relationships for our site were developed in summer 2007 before treatments began. 2009 sampling of sapwood thickness (with an increment corer) around sap flow probes has validated the allometric predictions of sapwood thickness. In 2011, we continued non-destructive approaches to re-evaluating these relationships to assess treatment affects on allometric relationships. In 2010, graduate student Amanda Boutz sampled small branches from pinon in all treatments. Using bud scars, Amanda measured annual growth increments on branches recorded and the leaf area associated with each increment. Her data clearly show differences among drought, irrigation and the two control treatments with respect to the leaf area and branch morphology. In 2011 and 2012, Amanda has developed methods for relating her branch scale measurements to tree level allometry. She is working on a manuscript to be submitted in spring 2013. Jean-Marc Limousin has also been using photographic methods (digital camera with wide angle or hemispherical fisheye lens) to estimate changes in canopy leaf area by a second method for comparison.

Plant responses to treatment and mortality patterns across plots: Our treatments have been effective, yielding substantial and consistent differences in soil water potential among treatments. These differences in soil water potential have been apparent in measurements of plant water potential. In drought treatments, anisohydric juniper reflecting the soil water potential differences more strongly than isohydric pinon. During the summer monsoon when water availability was at its greatest, plant water potential of trees in the drought plots was 35 to 52% lower, and photosynthetic rates that were 14 to 33% lower than trees in control plots. Supplemental irrigation resulted in a significant increase (46 to 69%) in plant water potential after irrigation compared to trees in the other treatments during dry seasonal periods. During these periods, irrigated trees were the only ones exhibiting net uptake of CO_2.

In drought treatments transpiration (E, measured via Granier sap flow) of both pinon and juniper was reduced in the precipitation removal plots relative to controls, most notably during the spring and monsoon moisture pulses. The critical transpiration rate leading to hydraulic failure (E_{crit}, estimated using the Sperry model) was much higher in drought-tolerant juniper than in pinon. Juniper E was conservative relative to these modeled limits, while pinon E approached hydraulic limits in both drought and control plots (Plaut et al., revised manuscript in review). Minimum pinon E was lower in the drought plot than in the control plots.

Consistent with pinon's small safety margins from hydraulic failure, stable isotope measurements of xylem water at peak drought show that xylem water is more enriched than any measured water source (including shallow soil, Yepez et al.) suggesting that at that time the trees may be hydraulically isolated from the soil, mostly likely because of cavitation in the roots.

In 2010 and 2011, graduate student Patrick Hudson measured branch hydraulic conductance across treatments using samples collected at predawn and midday. He found that there were no diurnal changes in hydraulic conductance in any treatment but that there were changes in hydraulic conductance, especially in the irrigation treatment. In 2012, Patrick measured vulnerability curves of stems and roots on every target tree across the experiment. Despite the sustained forcing applied to these plots, no shift in vulnerability to cavitation was observed in either species. This result suggests that in stems this trait is fairly conserved and that the study species are not able to exhibit acclimation of their hydraulic architecture.

Non-structural carbohydrate (NSC) sampling: During our last site review, we agreed that sampling of NSC would provide an important perspective on the carbon starvation hypothesis. NSC sampling was carried out throughout 2009 and has continued through 2011, with leaf and stem sampling at regular intervals and more intensive sampling of a broader range of tissues on two dates. During 2011, we completed analysis of our backlog of NSC samples dating back to 2009. While estimating whole plant NSC content is a challenging proposition because of variation across tissue types and our limited ability to sample the entire root portion of the plant, these data show interesting patterns. The data show that the pinon trees that died on the hillslope plots in 2008-09 exhibited little seasonal accumulation of NSCs compared to control trees that survived. Moreover, the tissue level measures of NSC were not zero at the time mortality occurred.

Measurement of phloem function across treatments: Dr. Sanna Sevanto (LANL postdoc) conducted detailed measurements of xylem and whole-stem diameter to infer phloem function. She is currently analyzing these data for publication to consider the hypothesis that carbon starvation occurs not because of strictly stomatal limitations but as a result of limitations on transport of photosynthetic products, and changing sink strength.

Measurement of leaf level gas exchange: Jean-Marc Limousin's 2010/2011 data on leaf gas exchange have also revealed acclimation in pinon and juniper subjected to irrigation and drought. Juniper exhibited large differences in not only the water potential associated with stomatal closure but the maximum photosynthetic rate during periods of high water availability. Gas exchange rates were always higher for juniper than pinon and there was always a clear hierarchy among the treatments, with the highest stomatal conductance and highest rates of photosynthesis and transpiration occurring in irrigation plots, lowest rates in drought plots and intermediate in the control and cover-control plots (Limousin et al, in review). In pinon there was a slight decrease in the water potential at stomatal closure but the maximum photosynthetic rate at high water potential decreased. This has the paradoxical effect of still restricting gas exchange under drought while reducing the maximum rate of gas exchange when water is available.

Reference watering experiment (Sept 2012): Several streams of data have provided evidence that acclimation has occurred in irrigation and drought plot target trees but such acclimation is difficult to assess when all plots never experience similar conditions. In Fall 2012, we sought to implement a "reference watering", in which we designed gravity flow irrigation systems for a single tree with the intention of applying a 50 mm equivalent irrigation. The goal of this treatment was to saturate soil moisture at the same time and to the same degree across all treatments to evaluate differences in the ability of trees to respond to these favorable conditions due to accumulated changes under different treatment regimes. Less than one week before this event, after all required water was positioned at the site, the site received over 90 mm of precipitation in a one hour period (25% of mean annual precip). This enormous event, combined with our water addition, produced very high water availability and the response of target trees was measured for three weeks after the storm/irrigation. Data analysis is ongoing but our preliminary evaluation shows clear differences in sap flow response suggesting underlying changes in allometry, leaf area and leaf physiology. A manuscript from this manipulative experiment is under preparation.

Fungal analyses: We have been collaborating with Don Natvig (UNM Biology), Paulette Ford (USFS, Rocky Mountain Research Station) and postdocs Andrea Porras-Alfaro and Joanna Redfern to understand as much as possible about the fungal component of the mortality cycle. These collaborators are funded by USFS to study the role of Ophiostoma fungi in pinon-juniper woodland. This group is currently engaged in an inoculation experiment to understand the progression of Ophiostoma infection in pinon. Our spraying plots will also address the question of the influence of the fungal infection on the trajectory of each tree with respect to hydraulic limits and stomatal limitations leading to carbon starvation.

Modeling analyses: The large scale of the manipulations and the long-duration of the study have resulted in high demand for the data from this study for use in model evaluations at a variety of scales from detailed plant level models to earth system models (Sperry et al 2016, Petrie et al. 2015, McDowell et al. 2015, MacKay et al 2015, Gentine et al 2015, Gustafson et al. 2015, McDowell et al 2013, and Plaut et al. 2012).

Products developed under this award.

Publications:

Peer reviewed publications:

Sperry, JS, Y Wang, B Wolfe, DS Mackay, W Anderegg, NG McDowell, and WT Pockman. 2016. Pragmatic hydraulic theory predicts stomatal responses to climatic water deficits. **New Phytologist.** doi: 10.1111/nph.14059.

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McDowell, NG, WT Pockman, CD Allen, DD Breshears, N Cobb, T Kolb, J Plaut*, JS Sperry, A West, D Williams, EA Yepez. 2008. Tansley Review: Mechanisms of plant survival and mortality during drought: Why do some plants survive while others succumb to drought? **New Phytologist.** 178: 719-739.

Data:

The key datasets collected during this project are freely available to the scientific community and the public via the LTER Data Portal:

Pockman, William; McDowell, Nathan (2016-03-29): *Ecosystem-Scale Rainfall Manipulation in a Pinon-Juniper Forest at the Sevilleta National Wildlife Refuge, New Mexico: Soil Temperature Data (2006-2013).* Long Term Ecological Research Network. http://dx.doi.org/10.6073/pasta/c7b878960a8cecac3c9ecd0552c53fa1

Pockman, William; McDowell, Nathan (2016-03-28): *Ecosystem-Scale Rainfall Manipulation in a Pinon-Juniper Forest at the Sevilleta National Wildlife Refuge, New Mexico: Water Potential Data (2006-2013).* Long Term Ecological Research Network. http://dx.doi.org/10.6073/pasta/4c7b3696c3101375fbde251622748738

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