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Intraspecific variation of drought tolerance in *Pseudotsuga macrocarpa*

Introduction

Little is known about Bigcone Douglas-fir (*Pseudotsuga macrocarpa*), a rare conifer endemic to semi-arid environments in southern California's Transverse Ranges. The U.S. Forest Service is interested in developing a better understanding of this species' basic biology in order to inform their restoration of stands of *P. macrocarpa* following recent drought and fire-related mortality in Los Padres National Forest. The present study focuses on characterizing the drought tolerance of intraspecific populations of *P. macrocarpa*. In other conifer species, drought physiology can vary between local populations along elevational and latitudinal gradients due to ecotypic variation; it is likely that the same is true for *P. macrocarpa.* To best inform the Forest Service's choice of *P. macrocarpa* populations to source seeds from when replanting, we will evaluate differential drought resistance in seedlings from seven different populations, as well as examine the drought responses of mature *P. macrocarpa* stands along an elevational gradient in Los Padres National Forest.

Background

Pseudotsuga macrocarpa is a rare member of a small genus that includes the better-known and far more widespread *Pseudotsuga menziesii*, or Coastal Douglas-fir. Unlike *P. menziesii*, which has a large range that spans many biomes but favors the damp forests of the Pacific Northwest, *P. macrocarpa* is endemic only to a small region in the Transverse Ranges of southern California, where it grows in steep, north-facing drainages among chaparral and Pinyon Pine (*Pinus monophylla*). Because of its small range, *P. macrocarpa* populations is at risk of local extirpation during episodes of regional environmental change. In 2007, the Zaca Fire burned 972 square kilometers near Santa Barbara, CA, causing severe fire impacts in many stands of *P. macrocarpa*. This, coupled with the several years of a record-breaking and climate change-driven drought (Williams et al. 2016) that followed the fire, caused high *P. macrocarpa* losses in nearly 40% of recently surveyed stands (Salladay et al., 2017). The U.S. Forest Service is heavily invested in replanting the stands that were lost to the fire but this requires information about the relative drought tolerance of different populations of *P. macrocarpa* in order to best inform their decisions on selecting seed sources given the projections for increased droughts in the region.

As part of an independent project in Plant Physiology, I collected preliminary data on the drought physiology and light response of a single population of *P. macrocarpa* from Los Padres

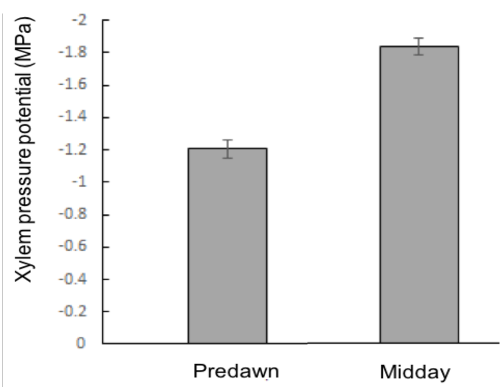

Figure 1. Predawn vs. midday field Ψ_p measurements of *P. macrocarpa*. The more negative midday Ψ_p values are typical, as plants tend to dry out over the course of the day due to stomatal conductance during photosynthesis. (Data from Plant Physiology independent project).

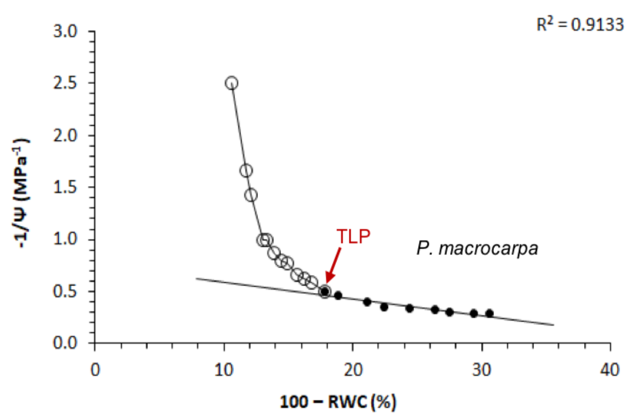

Figure 2. Pressure-volume curve of *P. macrocarpa* collected in the field. The turgor loss point of *P. macrocarpa* is -2.63 ±0.27 MPa. (Data from Plant Physiology independent project).

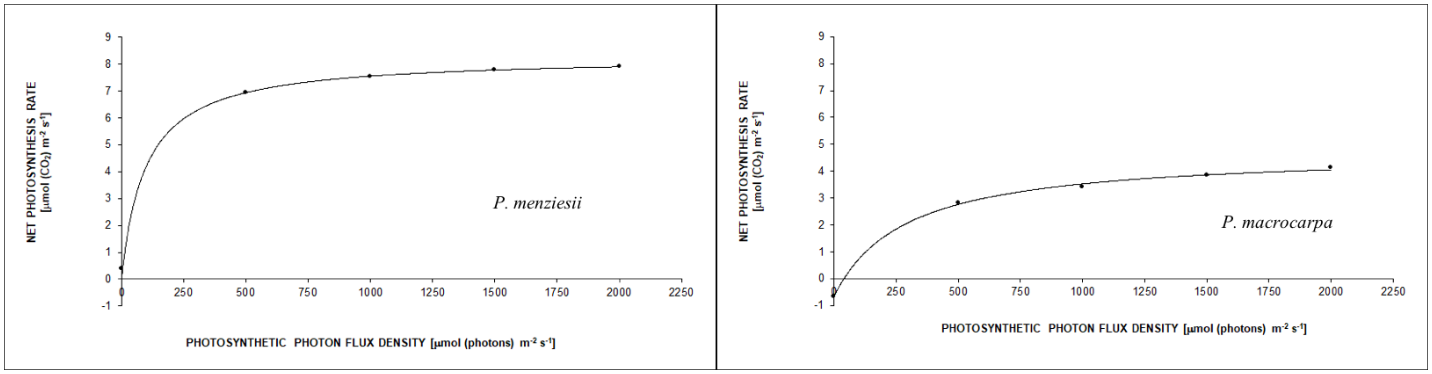

Figure 3. Light response curve of *P. macrocarpa* compared with that of the closely related *P. menziesii*. The higher light compensation point (69.8 ±13.7 vs. 6.3 ±4.5 µmol photons m^{-2} s^{-1}) and maximum saturation point (932.7 ±117.5 vs. 618.3 ±217.7 µmol photons m^{-2} s^{-1}) of *P. macrocarpa* are both consistent with a plant adapted to higher light level conditions. (Data from Plant Physiology independent project).

National Forest during the peak of the 2017 dry season. Field-based measurements of xylem pressure potentials (Ψ_p) over the course of a day (diurnals) showed a significant decrease in Ψ_p values from predawn to midday (Fig. 1). The average turgor loss point (TLP) of this population was similar to measured midday water potentials (Figs. 1 & 2), suggesting that *P. macrocarpa* individuals in this population approach a critical physiological threshold during the peak of the dry-season. In addition, measured light response curves (Fig. 3) show high light compensation and saturation points compared with those of the closely related *P. menziesii*, indicating adaptation of *P. macrocarpa* to high light environments. These preliminary data provide an important foundation for future investigations of drought tolerance and local adaptation in *P. macrocarpa* populations.

The Chinese conifer *Pinus tabuliformis* exhibits an elevational pattern in drought response, with seedlings from drier, high-elevation populations less affected by drought stress than low-elevation populations in a laboratory environment (Ma et. al., 2014). My research will help determine if *P. macrocarpa* populations exhibit similar intraspecific differences in drought tolerance would help better inform the U.S. Forest Service's efforts to restore *P. macrocarpa* stands in Los Padres National Forest.

Specific Aims and Hypothesis

Specific Aims:

Whether populations of *P. macrocarpa* differ in drought response is currently unknown. However, the restoration of stands recently damaged by fire and drought requires the US Forest Service to make decisions about potential seed sources. I will collect both lab and field-based data on *P. macrocarpa* drought resistance and response to summer drought conditions in order to provide the Forest Service with some of the data they need to choose appropriate *P. macrocarpa* seed sources.

Hypothesis:

Seedlings sourced from populations of *P. macrocarpa* that grow in drier environments, such as those at lower elevations, will exhibit greater drought resistance, including less negative pressure potentials and higher photosynthetic rates under drought conditions.

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Methods

Description of proposed ecophysiological measurements:

Xylem pressure potential (Ψ_p) refers to the directly measured water potential of a freshly cut tree stem. A more negative Ψ_p value indicates a more drought stressed plant. It is most frequently found using a pressure chamber. Xylem pressure potential measurements taken at intervals over the course of a day are useful in determining how much drought stress on a plant increases from predawn to midday (Fig. 1), while turgor loss point measurements (TLP) are used to determine drought resistance of the individual. The TLP is the first point on the linear portion of a pressure-volume curve (Fig 2). A pressure volume curve is a plot of -1/ Ψ_p by percent water content of the stem; it is found by measuring the Ψ_p as a stem dries out. The Ψ_p decreases at first exponentially, and then linearly once the cells are dried to the point where they lose turgor pressure. The first point on the linear portion of the curve is the TLP.

Stomatal conductance is the rate of water vapor exiting a leaf. A higher stomatal conductance indicates that the stomata are more open, and usually implies a higher photosynthetic rate. It is found using a leaf porometer.

Chlorophyll florescence is the fraction of light that, when shone on a dark-adapted leaf, is fluoresced back by Photosystem II. A value below 0.6 is generally considered indicative of an unhealthy leaf.

Photosynthetic rate is the rate of CO_2 production per leaf area (Fig. 3). It is found using an infrared gas analysis system, in this case a Li-Cor.

Dry mass is the total mass of the plant (including roots) after drying in an oven. Shoot mass and root mass will also be taken separately.

Lab-based methods:

The laboratory experiment will be conducted on 1–2 year old potted *Pseudotsuga macrocarpa* seedlings. 210 seedlings total will be obtained from the U.S. Forest Service, with 30 seedlings per population. There seedlings are sourced from seven populations at varying locations and elevations in the Transverse Range: Cleveland NF (seed zone 998, elevation 4,000-4,500 ft.), Angeles NF (seed zone 993, elevation 4,501-5,000 ft.), Angeles NF (seed zone 993, elevation 5,001-5,500 ft.), Angeles NF (seed zone 993, elevation 5,501-6,000 ft.), Angeles NF (seed zone 993, elevation 6,001-6,500 ft.), Angeles NF (seed zone 993, elevation 6,501-7,000 ft.), and San Bernardino NF (seed zone 994, elevation 5,001-5,500 ft.). The two treatments are drought and control. 5 seedlings/ population/ treatment will be used for non-destructive measurements throughout the experiment, while 5 seedlings/ population/ treatment will each be sampled at one of the three measurement periods: baseline, moderate drought, and severe drought. The 5 seedlings/ population/ treatment used for non-destructive measurements throughout will be used for the final destructive measurement after rehydration.

All seedlings will be immediately transplanted into pots containing identical potting soil and allowed to acclimate to greenhouse conditions, with watering 3 times per week for at least two weeks. At this point, baseline measurements will be taken. All trees in all groups will be measured in height and diameter at stem base. Non-destructive physiological measurements—stomatal conductance, gas exchange rate will be taken from all seedlings. Destructive physiological measurements—xylem pressure potential, dry mass, trunk xylem embolism—will be taken from the appropriate seedlings.

The drought group will then have water withheld for 6 weeks; the control group will continue to be watered 3 times/ week as before. At 3 weeks into the drought period, considered "moderate drought," and at the end of the drought period, considered "severe drought," all physiological measurements will again be taken for both the drought and control groups.

After the 6 weeks of drought, the seedlings will go through a 2-week "recovery period" during which they are once again watered 3 times/ week. At the end of this period, the final physiological measurements will be taken.

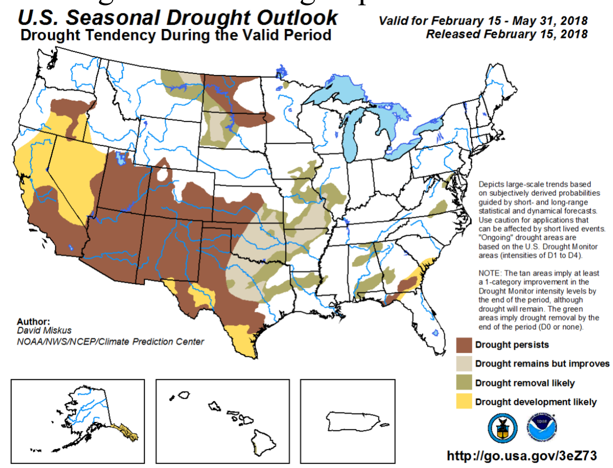

Figure 4. Seasonal drought outlook projecting persistent drought conditions across much of the southwestern U.S., including southern CA. Map from NOAA Climate Prediction Center: http://www.cpc.ncep.noaa.gov/

Field-based methods:
A comparative field experiment will examine the response of wild *P. macrocarpa* populations to seasonal drought. The seasonal drought conditions of summer 2018 are predicted to be extreme (Fig. 4). Los Padres National Forest, to date, has only received 1.5 in. of rainfall this winter, compared to the average 16 in. (weather.com). This field study will provide a comparison of drought responses in mature *P. macrocarpa* stands to understand how effectively the lab experiments with seedlings reflect drought responses in mature, wild populations.

During the months of June, July, and August, physiological measurements of four designated *P. macrocarpa* populations at varying elevations in Los Padres National Forest will be taken. These measurements will include stomatal conductance, xylem pressure potential, and gas exchange, all of which will be measured several times over the course of a day (diurnals). TLP measurements will also be performed. Abiotic factors will be monitored at the highest and lowest elevation sites using a Atmos 41 weather station, which measures air temperature, humidity, barometric pressure, wind speed and direction, solar radiation, and precipitation. These monthly measurements will allow us to understand the response of wild *P. macrocarpa* populations to seasonal drought and to better contextualize the laboratory data.

Predicted outcomes and alternatives, and impact
We expect that plants from drier regions and elevations will display increased drought resistance and lower drought stress under dry conditions compared to those from wetter regions and elevations due to local adaptation. Such an outcome would suggest that US Forest Service's restoration efforts may want to consider local adaptation to drought when selecting seed sources for replanting. Alternatively, if no difference in drought resistance/response is detected, it would suggest that variable drought tolerance is not as important of a consideration for these decisions.

Both outcomes would be vital to informing the Forest Service's replanting efforts and helping us better understand the drought physiology of a species likely to be heavily impacted by drought as the climate continues to warm.

Budget

Atmos 41 all-in-one weather stations (2)	$1500
($3500, but additional costs to be covered by Ramirez lab)	
Total	$1500

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