The BAR Project and SDSU Oak Resilient Restoration update

In 2020, we started with 1400 acorns collected in the Burn Area Rehab (BAR) location. A small amount was also collected for SDSU. Our Oaks will be returning in January after research is complete.

Although seeds were collected and propagated, the result was not successful. The reason could have been for many factors. Especially depending on the species of Oak. In the studies it has proven that *Quercus agrofilla* has a better successful rate compared to the *Quercus Engelmann*. Which we will be learning to identify the two.

This year's collection will be slightly different, we can expand our picking area and we will have funding for training purposes and possible irrigation materials.

Our Public Meeting on September 16th was supposed to include Dr. Lluvia Flores-Renteria, from SDSU, for a short presentation but with technical difficulties she was not able to join us. The material below introduces a quick demonstration of the Oak Resilient Restoration Research project.

Oak Resilient Restoration Research Update

September 2022

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"Background In the summer of 2020, the Climate Science Alliance, the Alliance's Tribal Working Group and researchers at the University of California, Riverside and San Diego State University (SDSU) kicked off a new collaborative project, "Resilient Restoration: Advancing Ecological, Cultural, and Community Resilience with Tribal Nations in Southern California," funded by California's Strategic Growth Council. The goal of this project is to promote Tribal resilience by developing knowledge and supporting actions to preserve the ecosystems and species that are integral to Tribal culture and communities. The research associated with the project will advance understanding of the impacts of climate change on a suite of native plant species in southern California, with a particular focus on oaks. Given the cultural and ecological importance of southern California's oaks to Tribal communities, the research team at SDSU partnered with several Tribal communities to conduct field- and greenhouse-based research to inform strategies to protect and restore oaks. A key component of this work is understanding what makes individual oak trees or populations of oaks resilient to climate stressors like extreme heat or drought. To accomplish this, we focused on resilience in two oak species, coast live oak and Engelmann oak. This research involved collecting acorn and leaf samples on ancestral and Tribal reservation lands and testing drought tolerance of oaks with greenhouse experiments to evaluate how different oak lineages or populations respond best to different watering regimes. For example, oaks from drier areas in the east may do

better (higher survival rates, faster growth rates) under drought stress than those from coastal zones. Using the results of this research in combination with traditional practices and knowledge about stewardship of oaks, we hope to collaboratively develop restoration planning strategies to protect these species now and in the future. Research Partnership Details To begin this work, we sought Tribal partner assistance to collect coast live oak and Engelmann oak acorns to establish greenhouse plantings and oak leaves for species identification and sequencing to examine the lineage of different samples we plant in the greenhouse. Specifically: ● We worked with Tribal partners to collect these samples in several locations ● We requested approval to gather location information for the samples that were collected. This information is only being used with great discretion during analysis and is being protected from any unauthorized release (see additional details below). ● All results and information from this work has been approved to be shared with Tribal partners who have contributed samples or are part of the Tribal Working Group. ● We have invited and continue to welcome any Tribal members and staff to visit the lab and greenhouse to participate in the research process and discuss greenhouse cultivation practices with staff at SDSU. ● We plan to return any surviving and viable greenhouse plantings to Tribal lands once the project has been completed. ● Once surviving, viable seedings are returned, we are also open to working with Tribal partners to create a common garden experiment to grow the oaks in local communities to help us all better understand how climate change affects them. Data and information protection We recognize that oaks hold a unique and significant place in Tribal culture. We honor and respect the sensitive nature of sharing these samples and locations with our team and have identified the following measures we are continuing to take to protect this information: ● We will not disclose the location of gathering sites with anyone beyond the researchers conducting the work ● We will use unique identifiers for samples collected on tribal lands and store location information separately to protect these sites ● Site specific information will never be shared with the granting agency nor in any public project materials or written reports for the project ● Any maps prepared for reports will obscure sites by offsetting points or representing collection sites in generalized zones ● Photos of sampling sites will only be shared with non-Tribal audiences with prior Tribal permission Acorn collection."

This information was shared by SDSU research team

SDSU Seed Collection Protocol

1) We aimed to collect acorns from three areas per site sampling from approximately 10 trees per single area. At each site we attempted to collect 10 trees of *Quercus agrifolia* and 10 *Q. engelmannii* if both species were present.

2) Each sampled tree was spaced at least 100 ft from other sampled trees

3) GPS coordinates were taken at the base trunk of the tree and recorded in the Survey12

4) All acorns from one tree were collected in a paper lunch bag, which was labeled with tree ID

   a) We used the first letter of both genus and species and then the first 3 letters of the reservation and a number in sequential order.

   b) For example, QA will be the label of the first *Quercus agrifolia* collected at Reservation, QE, will be the label of the first *Q. engelmanii*

5) We collected at least 15 acorns per tree.

   a) Acorns were collected directly from the tree. Acorns were not collected from the ground as those acorns could not reliably be linked back to the maternal tree and could potentially have reduced viability.

   b) Acorns that were black, had dark discolorations or holes are usually bad, and were not included in the collection sample.

c) Yellow to greenish acorns or brown were preferentially kept.

6) Two small branches 6 to 9 inches long with healthy leaves were collected to be included in genetic analysis.

a) The branches were smaller enough that they fit in the paper bags.

b) One of these branches had new growth and the other branch needs to have fully developed leaves to use for identification

7) Acorns and branches from each tree were placed in an individual paper bag and each bag was uniquely labeled

8) Diameter at breast height measurements were taken and included into the Survey123 app, as well as pictures of each tree and other relevant visual observations such as co-occurring species

SDSU Seed Germination Protocol

We applied the most successful protocol based on previous germination trials (See video https://youtu.be/E7cfJJqdD94).

1) All seeds were taken to the Evolutionary Plant Ecology laboratory at San Diego State University. All acorns were kept separately by maternal identity at all stages.

2) We cleaned all acorns using a diluted 5% bleach-water solution[1] to reduce the risk of introducing any pathogens from the various field sites into the greenhouse that might affect germination or survival during the experiment. The cleaning process involves briefly suspending acorns in the bleach-water, which should weed out any "floaters". Floating acorns are not viable, so we only retained sinking acorns.

3) The acorns were rinsed with running water to remove any of the remaining diluted bleach solution.

4) During the cleaning process, we examined acorns for any visible scars, holes, or pests and removed those that had any signs of damage.

5) After we cleaned the acorns, we used a cold stratification step to replicate the natural over-winter process that many seeds go through in a soil bank. All seeds were wrapped in paper towels and sprayed with water to dampen but avoiding soaking. The paper towels wrapped acorns were then placed in plastic bags. We refrigerated the bagged acorns for at least 60 days. This mimics what acorns experience in their natural environment during winter.

a) All acorns were placed in open bags in the refrigerator (4 °C) as acorns have high fat content and need to respirate (i.e., do not close the bag).

b) Paper towels were monitored weekly and sprayed water when needed to maintain moisture.

Going into the Future (Fiscal Year 2023)

CEPA would like to start gathering acorns in late October 2022.

Stage #1 October: scout out which trees to collect from. Watch the development of acorns. Have consent forms completed by participants. Continue working with Lluvia to help identify *Quercus agrifolia* and *Quercus engelmanii* Oaks.

[1] The intent behind the use of diluted bleach solutions in greenhouse and nursery environments is to avoid the introduction of pathogens into those environments that could affect the outcome of an experiments or risk the spread of pathogens beyond the greenhouse, which could pose a threat to wild populations of native plants.

Stage #2 Late October – November: collection of acorns. Add location on GPS, as well as the date and number of acorns. Keep in paper bag until tagged and individually wrapped and stored in refrigerator to begin germination process. There will be a significant amount that will be planted once the color turns golden brown without being refrigerated.

Stage #3 it takes up to 6-8 weeks for the germination. During the 60 days we will continue to observe and dampen the paper towels as necessary. Once we see root development, we will carefully pack them into soil and store them in CEPA's greenhouse. This will be sometime in January 2023.