Point Blue Conservation Science

Inoculant-Supported Restoration: A Technical Report

January 2022

Jessie Ditmore, Students and Teachers Restoration a Watershed Project Manager, measures the height of a coast live oak (Quercus agrifolia)
Cover Photo: A planted streambank in Sonoma County

Inoculant-Supported Restoration

Technical Report -- January 2022

Prepared by

Point Blue Conservation Science

Kristen Dybala PhD Isaiah Thalmayer

Thomas Gardali Chelsea Carey PhD

With funding from

Wildlife Conservation Society Climate Adaptation Fund

Suggested citation:
Dybala, K.E, Thalmayer, I., Gardali, T., and Carey, C.J.* 2022. Inoculant-Supported Restoration: A Technical Report. Point Blue Conservation Science, Petaluma, CA.

*Corresponding author: ccarey@pointblue.org

This is Point Blue Contribution No. 2392

Website link:
www.pointblue.org/ISR

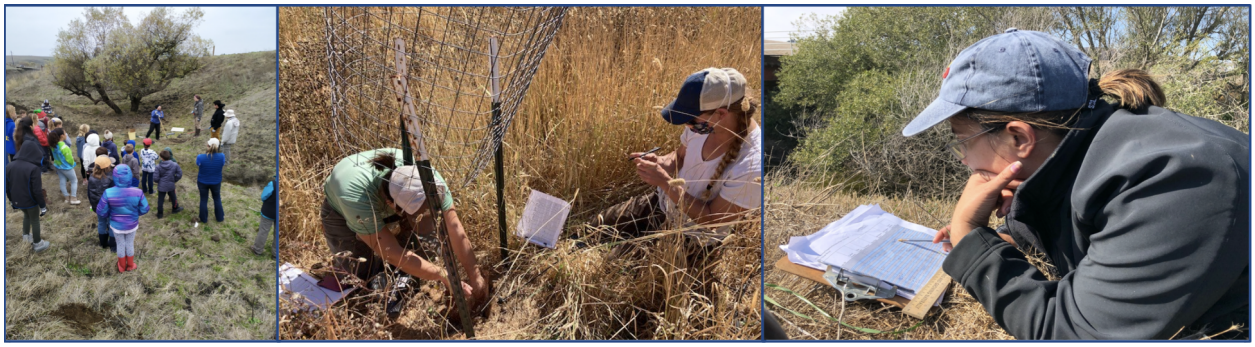
ACKNOWLEDGEMENTS

We would like to thank the staff and participants of Students and Teachers Restoring a Watershed (STRAW) for their incredible help in the field. In particular, we'd like to thank Jessie Ditmore, Alison Pollack, Josh Nuzzo, Leia Giamastiani, Lara Stagner, Drew Mealor, and John Parodi for their help in conducting the I-SR experiment, from inoculant collection through monitoring. We'd also like to acknowledge and thank Bridget Hilbig at Weber State University for conducting analysis of % root mycorrhizal colonization as part of this project. We're grateful to the landowners and land managers that supported the field research, including Five Springs Farm, Ocean Breeze Farm, Sonoma County Agricultural Preservation and Open Space District, Goldridge Resource Conservation District, and Sonoma Water.

A planted streambank in Sonoma County, California

ABSTRACT

In this report, we seek to introduce riparian restoration practitioners to a climate-smart restoration strategy we call Inoculant-Supported Restoration (I-SR). This strategy aims to restore relationships between riparian trees and their soil microbial communities, especially targeting beneficial ectomycorrhizal fungi. Here we describe the need for climate-smart restoration strategies, the rationale behind I-SR as a climate-smart strategy, and the evidence from a recent field experiment that this approach can improve the establishment and early survival of planted trees. We also provide detailed instructions on how to incorporate I-SR into riparian restoration projects.



Point Blue staff and STRAW students help to implement and monitor an I-SR project in Sonoma County.

TABLE OF CONTENTS

Inoculant-Supported Restoration (I-SR) as a Climate-Smart Restoration Strategy

Riparian ecosystems are a high priority for conservation and restoration in California because they can provide many important benefits, including water quality maintenance, carbon storage, and important habitat for fish and wildlife (Dybala et al. 2017). To ensure that restoration efforts are successful in providing these benefits over the long-term, it is critical that designs be climate-smart (Seavy et al. 2009). Climate-smart restoration projects are those that:

- consider projected future climate conditions and the vulnerabilities of the project to climate change
- identify ways to reduce those vulnerabilities
- work to improve the long-term resilience of the restored area

For example, much of California is projected to experience more frequent heat waves and extreme droughts, threatening the survival of many riparian plant species (Ackerly et al. 2018). In response, climate-smart restoration designs may include more heat- and drought-tolerant species in the planting palette, and/or ensure greater genetic diversity in the source material to improve the odds of survival and resilience to future disturbance (Parodi et al. 2014).

An important, but often overlooked strategy for climate-smart restoration lies in restoring the belowground community of soil microbes. In particular, many of the tree species commonly planted as part of riparian restoration efforts are associated with specialized ectomycorrhizal fungi that colonize their roots (Brundett and Tedersoo 2020). In exchange for carbon, these fungi help trees access water and nutrients in the soil and may play an important role in tree establishment and survival, particularly through periods of extreme heat or drought (Gibert et al. 2019). However, these specialized fungi may no longer be present in the soil of many restoration sites, especially if there have been no tree roots for them to colonize for many years (Nara 2008). Therefore, intentionally inoculating trees with these fungi to restore this relationship can be an effective strategy for improving the initial establishment and survival of planted trees as well as long-term growth and resilience to climate change.

Plants that associate with arbuscular mycorrhizal fungi (AMF) are not targets for I-SR of riparian areas because unlike ectomycorrhizal fungi, AMF associate with herbaceous plants and are more likely to already be present at the restoration sites.

Microbial inoculation has been demonstrated to be effective in many restoration contexts globally (Wubs et al. 2016, Neuenkamp et al. 2019). By specifically harnessing drought- or heat-adapted microbes, it may be possible to improve the odds of tree survival and restoration success via inoculation even as extreme conditions become more common (Valliere et al. 2020). In the case of riparian

restoration, this entails inoculating trees with fungi collected from a site that is already experiencing warmer and drier conditions. We call the strategy of applying local or drought-adapted inoculum Inoculant-Supported Restoration (I-SR). As with most restoration practices, research continues to play a necessary and pivotal role in better understanding and improving I-SR across diverse environments—and protocols for applying inoculation as part of riparian restoration in California are needed. We provide here an account of our findings of an I-SR field experiment and offer detailed guidance on how to add inoculants to the restoration process. We encourage restoration practitioners to consider experimenting with this strategy in their future riparian restoration projects.

Evidence for the Effectiveness of I-SR

Between 2019 and 2021, we conducted a multi-site field experiment in Marin and Sonoma counties, California, to test the effectiveness of I-SR for improving the establishment and initial survival of trees planted during riparian restoration. As a primary objective of this experiment, we sought to compare the effectiveness of inoculant sourced locally from either 1) a site with a similar climate to the restoration site ("local inoculum") or 2) from a hotter and drier site that currently has a climate analogous to the future climate projected for Marin and Sonoma counties later this century ("drought-adapted inoculum"). We assumed soil inoculum from the hotter and drier site would contain ectomycorrhizal fungi that were better adapted to, and tolerant of, drought conditions.

Photo 1. *(Left) Three acorns were planted within each oak replicate, a common practice for Point Blue restoration projects. (Middle) Willows were planted as cut sprigs. (Right) Douglas fir were planted as saplings in sterilized potting medium.*

We tested four tree species across three restoration sites, including coast live oak (*Quercus agrifolia*), valley oak (*Q. lobata*), arroyo willow (*Salix lasiolepis*), and Douglas fir (*Pseudotsuga menziesii*), although not all species were planted at all sites. For

each tree species within each site, we planted 24 trees, split into three treatments of 8 trees each: control (uninoculated), local soil inoculum, and drought-adapted soil inoculum. Oaks were initially planted as 24 sets of 3 acorns at each site, and culled after germination as needed (Photo 1). Willows were planted from cuttings, and Douglas firs were planted as saplings from containers (Photo 1).

Over the following two years, we documented the germination of oak acorns, the survival of all individual trees, and the colonization of tree roots with ectomycorrhizal fungi. For each tree species, we then conducted Bayesian analyses to estimate the probability of a difference in germination, survival, and root colonization rates between each soil inoculum treatment and the controls, as well as the magnitude of those differences.

For some of the tree species, we found strong positive effects of the drought-adapted soil inoculum in comparison to the control treatment. The odds of both coast live oak and valley oak acorns germinating with the drought-adapted soil inoculum were double that of controls, while the odds of coast live oak and arroyo willow survival were 3.3 and 4.1 times higher than controls, respectively (Figure 1). In addition, both coast live oak and arroyo willow had significantly higher proportions of their roots colonized by ectomycorrhizal fungi than controls. The effects of treatment with the local soil inoculum were less statistically significant, but may have improved the germination rates of valley oak acorns and their subsequent survival over two years (Figure 1). Finally, there was no evidence for any negative effects of either the local or drought-tolerant soil inoculum on the survival of any of these four species.

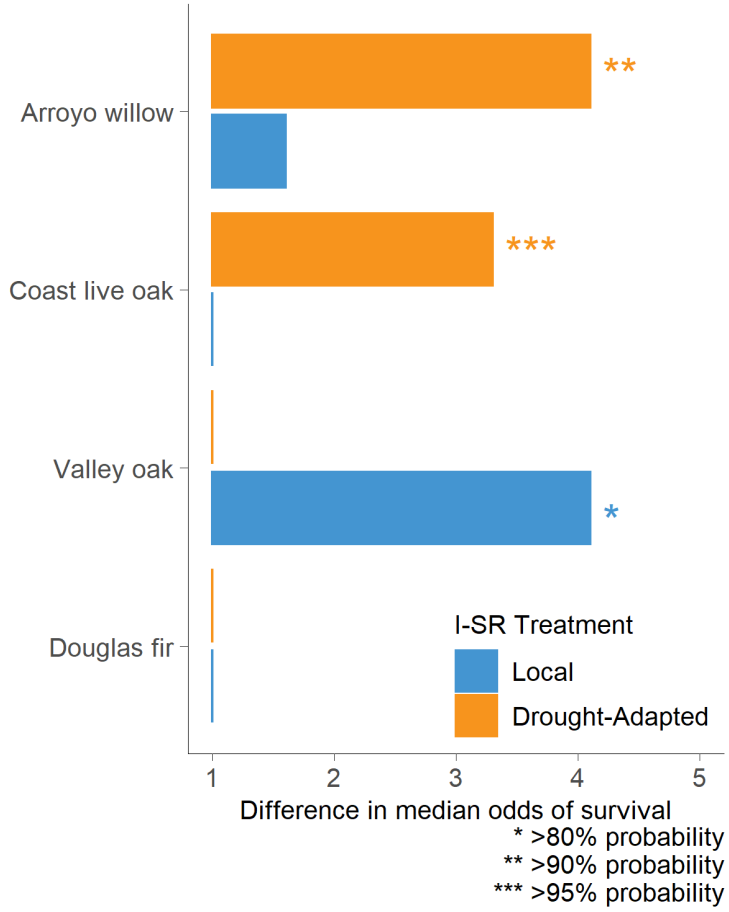


Figure 1. *Effects of I-SR treatments on odds of survival compared to uninoculated controls.*

Our results indicate that I-SR is a promising climate-smart restoration strategy with the potential to improve at least

the initial establishment and survival of several riparian tree species. We recommend further research and experimentation with I-SR in other regions of California, with other species, and over a longer period of time to better understand the conditions under which this strategy is most effective.

Protocol for Conducting an I-SR Project

In this section, we provide protocols describing how to conduct I-SR as part of riparian restoration projects and provide guidance on how to monitor the results.

Inoculant Collection

Finding the right soil inoculant requires careful consideration and comparison of site conditions at the restoration and collection sites. To locate and collect inoculant for I-SR, follow the steps below.

Materials and Supplies

- Computer
- GPS
- Soil knife or trowel
- Gallon-size ziploc bags and sharpie
- 10% bleach or 70% isopropyl alcohol and tissue

Step 1: Identify restoration and inoculant collection sites.

- The first step to identifying appropriate collection sites is to determine where you will be conducting restoration. Sites that have been devoid of ectomycorrhizal host trees for a long time (e.g., decades), and which do not have surrounding in-tact riparian forests, will be the most in need of I-SR intervention. It will also be important at this step to decide on the planting palette and determine whether any ectomycorrhizal host trees are planned for inclusion.

Common riparian ectomycorrhizal host plants include those found in the genera Alnus, Corylus, Quercus, Populus, Pseudotsuga, and Salix.

- Next, determine whether you'd like to find collection sites that are currently experiencing a similar climate to the restoration site, or collection sites that represent future conditions projected under climate change. In either case, you can use resources like CalAdapt and PRISM to explore and download climate data. CalAdapt provides the current and projected range of average annual precipitation and minimum and maximum temperatures for any location in California. Whether you prefer to use the current or projected values, these statistics can be used to define the "climate envelope" within which collection sites should fall. You can continue to use CalAdapt to compare the climate envelope to the current average climate statistics at

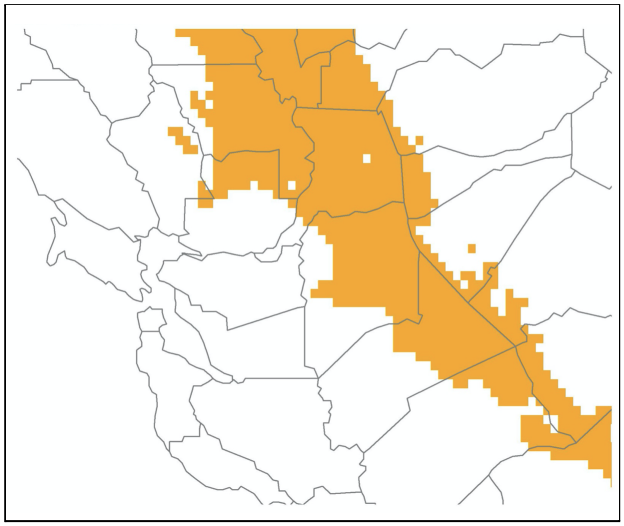


Figure 2. *Areas currently within the climate envelope projected for our I-SR study areas in Marin and Sonoma counties.*

candidate collection sites that have already been identified, or for a more systematic approach, you can download climate data maps from PRISM and use GIS software to map all of the nearby areas that currently fall into the desired climate envelope (Figure 2).

- Within the chosen climate envelope, we also recommend finding collection sites that have a similar soil type (at minimum the same soil order and texture classification) as the restoration site. To do this, applications such as UC Davis' Soil Series Extent Explorer, SoilWeb (computer or phone app), SoilWeb Earth, or Soil Properties can be helpful. Assessing texture can be done qualitatively in the field using the texture-by-feel method.
- Finally, choose collection sites that are relatively mature and healthy, that you have access to, and that have the target tree or shrub species of interest. For instance, if you are going to plant coast live oak, find collection sites that have coast live oak from which to collect soil inoculum.

Step 2: Visit the collection site to collect inoculum.

- At the collection site, find 3-5 individual trees per target species from which to collect soil inoculum. Collecting from 5 is ideal, but at minimum collect from 3. So, for instance, if your project includes both coast live oak and valley oak, collect soil from beneath 3-5 coast live oak individuals and 3-5 valley oak individuals. Focus on those individuals that don't show any obvious signs of stress or disease.
- At each individual tree, collect soil twice: once upslope and once downslope, approximately 1 pace away from the tree's trunk (you want to remain beneath the tree's canopy if possible; Figure 3).
- Once you've identified your sampling location, gently remove big pieces of litter (i.e., full leaves) but do not brush away the topsoil as this is where you will find most of the ectomycorrhizal fungi of interest.
- Use a hand trowel or soil knife pre-sterilized with 10% bleach or 70% isopropyl alcohol to collect

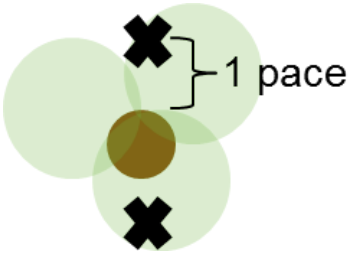


Figure 3. *An aerial view of an illustrated tree trunk, canopy, and sampling locations.*

soil from the surface down to 15 cm depth. Excavate a hole that is about 10 x 10 cm in area and place in a pre-labelled ziploc bag.
- Combine both samples from the same tree into one bag, but do not combine samples across trees.
- If possible, keep a written list of understory species present and take a GPS location at each tree. This could be important if you need to go back and get any more samples based on phytophthora results below.
- When collection is complete, keep samples sealed in the ziploc bags (do not air dry) at room temperature prior to pear-baiting. Storage at room temperature is especially important if samples were collected from hotter, drier sites and are being stored for relatively long (>2 weeks) periods. With enough time, cold storage could select against those heat-adapted fungi you are looking to include.

Phytophthora Testing

Phytophthora are pathogens that are responsible for killing plants throughout California. Perhaps most conspicuously, they are the culprits behind Sudden Oak Death (SOD). Recently, soil and water-borne *Phytophthora* species have been recovered from restoration sites across California. This is a concern since *Phytophthora* can spread to new plant hosts quickly upon introduction. Testing restoration plants and soil inoculum for the presence of *Phytophthora* using a pear-baiting method is an effective way to minimize the spread of this pathogen, and it is strongly recommended to include such testing as part of all restoration efforts. For I-SR, to store the soil inoculant and test it for the presence of phytophthora, conduct the following steps (which were adapted from the Phytosphere website):

Materials and Supplies
- 1-gallon potting containers
- 5-gallon buckets
- Gallon-size ziploc bags and sharpie
- 10% bleach or 70% isopropyl alcohol
- Unripe D'Anjou or other green pears
- Water (preferably deionized)
- Saran wrap (if conducting step 2)
- Paper plates

Step 1: Transfer soil inoculant to pre-sterilized potting containers.

- Pre-sterilize the potting containers using 10% bleach or 70% isopropyl alcohol
- Transfer each soil inoculant sample into its own pre-sterilized potting container. Be sure to keep each sample separate and all samples clearly, consistently labelled.

Step 2: If necessary, revive dormant phytophthora. (This step is not necessary if samples are already moist.)

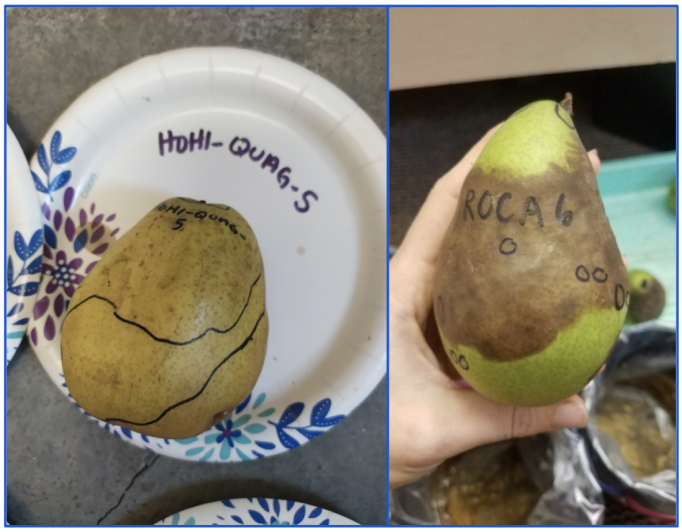
- If soil samples were collected during a particularly dry period, then any *Phytophthora* that are present may be dormant. To revive any dormant *Phytophthora*, gently add a small amount of water to each container so that the soil is moist but not saturated.
- Cover each pot with saran wrap to minimize evaporation, but do not form a complete seal so some airflow may still occur.
- If needed, repeat watering once a day to keep the soil moist.
- Allow soils to incubate for three days.

Step 3: Perform pear baiting.

- Line additional gallon-sized pots with ziploc bags.
- Place the gallon-sized pots containing soil from step 1 inside the pots lined with ziploc bags.
- On each unripe D'Anjou pear, lightly circle the existing blemishes with a permanent marker. This will help identify areas that were previously blemished versus those that develop blemishes through the pear baiting process. Do not remove the store sticker, as this may damage the pear.
- Place one unripe D'Anjou pear on top of the soil in each pot.
- Add water to the soil until the water level is above the soil surface and the pear is sitting in water (Photo 2).
- Let the pears sit for one hour, then slowly lift the soil pot out of the pot below, letting the water drain into the ziploc bag.

Photo 2. *(Left and middle) Pears sit on top of the soil, submerged in water, for one hour. (Right) After one hour, soil is left to drain in 5 gallon buckets.*

- Place the soil pot in a 5 gallon bucket to let it drain overnight (Photo 2) then transfer the soil to a clean gallon-size ziploc bag and store open to let some of the water evaporate. Close the bag if there are signs that the soil might be drying out (want to keep it at least field moist).
- Place the pears in the pots lined with ziploc bags, which are now filled with soil leachate, and let it sit for 3 days.
- After 3 days, remove the pears from the leachate and place on labelled plates.
- Let the pears sit for another 5 days, then evaluate the pears for signs of *Phytophthora* (Photo 3).



Do not confuse Pythium (a group of water molds closely related to Phytophthora) infection for Phytophthora. Pythium commonly infect pears, but can only infect a wounded site. In contrast to Phytophthora, Pythium create soft lesions that are water-soaked in appearance.

Photo 3. *(Left) A pear is left to incubate on a paper plate for 5 days. (Right) A pear that is infected with phytophthora shows signs of disease.*

Step 4. Collect and save the leachate.

- The leachate can be stored in the gallon-sized bags used in Step 3.
- Make sure the bags are labelled appropriately and closed fully for storage.

Step 5. Identify and report samples that show signs of *Phytophthora*.

- Assess pears for medium to dark brown lesions that are firm to the touch. Phytophthorsa species do not tend to cause softening of the pear during infection (Photo 3). See this link for numerous examples of *Phytophthora* infected pears.
- Send any pear that shows signs of Phytophthora for confirmation of infection by a diagnostic lab. The California Department of Food & Agriculture has a

Plant Pest Diagnostic Center that will accept baited pears, but there are other labs as well.

Step 6. Combine samples to create the final inoculant.

- Discard any soil or leachate samples that have been confirmed infected with *Phytophthora*. If too many samples are lost due to *Phytophthora* infection, you will need to consider re-collecting new soil samples and going through the pear-baiting process again to create the final inoculant.
- At minimum, combine all Phytophthora-free inoculum from the same target tree/shrub species, keeping the leachate and soil samples separate. If you are looking to inoculate multiple species, consider combining soil inoculum that was collected from beneath all species into one composite sample (Figure 4). This will ease the logistics of inoculant application in the field, and could potentially confer greater benefits to each restored plant by increasing the number of ectomycorrhizal fungal species introduced.
- Store the soil and leachate inoculum sealed at room temperature until use in the field (Photo 4).

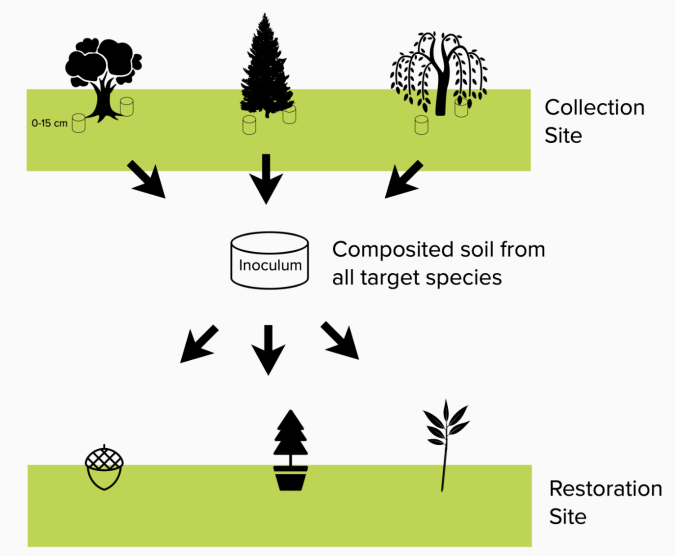


Figure 4. *Graphical illustration of inoculant collection, processing, and application that composites across tree/shrub species.*

Inoculant Application and Tree Planting

How you install your plants is going to depend on what species it is. For instance, planting willows typically occurs by inserting cut sprigs, whereas planting oaks is often done by burying acorns. Regardless of the plant species and installation approach, the method to add inoculum is going to be similar and will include adding soil and leachate from pear baiting to the bottom of the planting hole. To add inoculant as part of any restoration project, follow the steps below.

Materials and Supplies

- Soil and leachate inoculant
- Kitchen tablespoon
- 10% bleach or 70% isopropyl alcohol and tissue
- Metal tags and zip ties

- Supplies for plant installation (will depend on species and preferred methods)

Step 1. Prepare the planting site as usual.

- Get the site ready for installing plants as you normally would. This may include punching holes for willow sprigs, removing ground cover and installing seedling baskets for oak acorns, or digging a small hole for container plants. Whichever your method of planting, complete all the pre-planting steps, then advance to step 2.

Photo 4. *Soil and leachate inoculant ready to deploy in the field. The DI water on the far left was added to the untreated controls as part of the experiment.*

Photo 5. *Restoration supplies to complete an I-SR project in Sonoma County, California.*

Step 2. Add inoculant to each plant and complete plant installation.

- Once the site is prepared—but prior to placing the sprig, acorns, or container plant in the ground—you'll want to add the inoculum.
- Add no less than 2 tablespoons of soil inoculum and 1 tablespoon of inoculum leachate to the prepared site. Sterilize the tablespoon before use with 10% bleach or 70% isopropyl alcohol. Focus on applying the inoculant directly in the bottom of the planting hole, or otherwise adding it to the center of the planting location beneath where the plant or acorns will be introduced.
- Finish installing the plant as you would normally (Photo 5).

Monitoring Restoration "Success"

Monitoring an I-SR project can help you to gain a better understanding of project success and, if done appropriately (e.g., by comparing to uninoculated controls), can also promote greater scientific understanding of this approach across varying contextual gradients. There are a number of ways to approximate success, the most

direct of which include measuring germination (if applicable) and survival. Proxies for plant biomass and vigor can also provide indication of success both in the near- and long-term, although in our experiment we found the former metrics to be most discerning. We therefore focus on providing guidance on how to monitor germination and survival below.

Materials and Supplies

- Clipboard, pen, and paper
- GPS

Step 1. Locate the I-SR plants using GPS or other techniques.

Step 2. Determine whether each plant is living or dead, and record the information on a piece of paper (Photo 6). For oaks or other plants that were directly seeded, first record whether the acorns germinated. If multiple individuals were seeded per location, record how many of the total germinated. Existing monitoring frameworks, such as the Riparian Zone Monitoring Plan, can be used to help assess plant survival and vigor.

Photo 6. *(Left) A coast live oak has survived its first winter. (Right) A clipboard facilitates data collection of germination and survival.*

References

Ackerly D, Jones A, Stacey M, Riordan B (2018) San Francisco Bay Area Summary Report. California's Fourth Climate Change Assessment. Publication number: CCCA4-SUM-2018-005. Available from: http://climateassessment.ca.gov/regions/docs/20190116-SanFranciscoBayArea.pdf

Brundrett MC and Tedersoo L (2020) Resolving the mycorrhizal status of important northern hemisphere trees. Plant and Soil, 454: 3-34. https://doi.org/10.1007/s11104-020-04627-9

Dybala KE, Clipperton N, Gardali T, Golet GH, Kelsey R, et al (2017) Population and habitat objectives for avian conservation in California's Central Valley riparian ecosystems. San Francisco Estuary and Watershed Science 15: art5. https://doi.org/10.15447/sfews.2017v15iss1art5

Gibert A, Tozer W, Westoby M (2019) Plant performance response to eight different types of symbiosis. New Phytologist 222:526-542. https://doi.org/10.1111/nph.15392

Nara K (2008) Spores of ectomycorrhizal fungi: Ecological strategies for germination and dormancy. New Phytologist 181: 245-248. https://doi.org/10.1111/j.1469-8137.2008.02691.x

Neuenkamp L, Prober SM, Price JN, Zobel M, Standish RJ (2019) Benefits of mycorrhizal inoculation to ecological restoration depend on plant functional type, restoration context and time. Fungal Ecology 40: 140-149. https://doi.org/10.1016/j.funeco.2018.05.004

Parodi JJ, Giambastiani L, Seavy NE, Thalmayer IM, Lasky E, Gardali T (2014) A how-to guide and metadata for the Riparian Restoration Design Database for Marin and Sonoma counties. Point Blue Conservation Science, Petaluma, CA. www.pointblue.org/restorationtools

Seavy NE, Gardali T, Golet GH, Griggs FT, Howell CA, et al (2009) Why climate change makes riparian restoration more important than ever: Recommendations for practice and research. Ecological Restoration 27: 330-338. https://doi.org/10.3368/er.27.3.330

Valliere JM, Wong WS, Nevill PG, Zhong H et al (2020) Preparing for the worst: Utilizing stress-tolerant soil microbial communities to aid ecological restoration in the Anthropocene. Ecological Solutions and Evidence 1: e12027. https://doi.org/10.1002/2688-8319.12027

Wubs ER, van der Putten WH, Bosch M, Bezemer TM (2016) Soil inoculation steers restoration of terrestrial ecosystems. Nature Plants 2: 16107. https://doi.org/10.1038/nplants.2016.107