Sacramento Tree Foundation Acorn Collection Protocols

There are critical issues that must be considered when collecting native seed for the purpose of restoration/reforestation/revegetation. Our standards and protocols are designed to ensure that planting material developed by the Sacramento Tree Foundation is *genetically appropriate*, *genetically diverse* and has not been influenced by *unintended selection* due to the way the collection occurs. Additionally, special consideration must be taken to ensure that harvesting practices are compliant with local law, do not pose a harm to the natural communities where propagules are harvested, and will not result in materials infected by insects or pathogens.

1. Genetically appropriate, in this sense, is commonly understood to mean that the seeds used in a given project were collected from naturally occurring populations that are adapted to local conditions. For plants like oaks that hybridize indiscriminately with non-native oaks planted nearby, it is important to verify that the collection material (acorns) have not been produced through unintentional hybridization. Though the creation of unique hybrid oaks may be desirable for the landscape industry, it is not desired or appropriate within projects with the purpose of creating or enhancing native landscapes. The long-term implications of using novel hybrid oak trees instead of native oaks in natural landscapes could have significant and irreversible consequences to ecosystems and wildlife. We do not collect from trees that we know were human-planted or are within 2,000 feet** of non-native oaks of the same subspecies, making their acorns possibly hybridized.*

2. Genetically diverse is commonly understood to mean that seeds were collected in a way that ensures a representative sample of the genetic material present within any given population is retained. It is not appropriate, nor ethical, to collect large quantities of seed from a single plant or grove as this can lead to genetic erosion (loss of the ability to adapt in the future) within the future population. Also, the removal of too large a percentage of seed from an area can do significant damage to the wild population from which you are collecting or the wildlife that depends upon that food source.

   Many sources of native trees propagate their commercial product from only one or two parent trees which limits genetic diversity within their trees and does not represent differences resulting from a variety of local conditions. This limits the future evolutionary potential of our re-forested (planted) oak populations and significantly increases the likelihood of poor compatibility with soil or moisture regimes in the out-planting site. Our protocol is to ensure that our collection sites

cover all of the representative oak habitats in our planting area as well as a variety of soil types, moisture regimes, and elevations within those habitats.

In order to maximize genetic diversity in our source materials and avoid preventable future genetic erosion from occurring within our reforested sites, we collect relatively small quantities of seeds from as many separate locations and individuals as possible. Our protocol is to collect no more than 5% of the available acorns from no less than 15 to 25 unique locations within the region each season.

3. Arbitrary collection pressures are caused by individuals unintentionally influencing the genes captured by collecting only a portion of the available viable seed rather than a representative sample of all the seed available. For acorn collecting, the most common unintended selection is caused by acorn collectors only picking up the biggest size class of acorns. We collect all sizes of acorns as long as they are healthy. Though bigger seeds do result in more robust plants in the short term, the unintended result of this practice is the possible loss of genetic diversity; possibly evolutionarily important genes found only within the small acorns in the group. This will limit the variety of genes available to the next generation and potentially the ability to adapt to changing future conditions. Ideally, if you graphed out the size classes of a collection sample, the result should be a bell curve with limited numbers of extremely small and extremely large seeds as the outliers on both sides. Our protocol is to collect an equal percentage of all sizes of acorns, as long as they are healthy. (See acorn collecting guidelines for how to select healthy, viable acorns).

4. Potential for insect or pathogen contamination must be evaluated before a seed harvest is initiated and additional steps may need to be taken. For an acorn harvest, it is ideal to harvest seed directly from the tree, at a height of more than three feet. As we are aware that this ideal condition is rare, harvesting from the ground requires that we determine if contamination by insects or pathogens is likely. In areas known to be contaminated by *P. ramorum* or other non-native phytophthoras, post-harvest treatment will include bleach treatment. Bleach treatment will also be used when harvested acorns are visibly contaminated by moist soil or mud.

Seed Collection Protocol Recap

- Select collecting locations that encompass all the variety in habitat, soil type, moisture level and elevation that occur in your planting sites.
- Maximize the number of collecting locations/individual trees and minimize the number of seeds collected at each one (no more than 5% of what is present).

- Do not collect from populations that may be in contact with foreign genes or from populations that were established during previous restoration/revegetation project.
- Try to avoid arbitrary selection by collecting a sample of all the seeds available. Do not take only the biggest/smallest seeds, try to collect from multiple ripening events (if applicable), etc.
- Be aware of the impact on the ecosystem caused by the removal of seeds/ propagules.
- Check your harvesting location for known contamination by pathogens such as phytophthoras. Either avoid harvesting in these areas or perform appropriate post-harvest sanitation.
- For non-oaks, understand the biology of your plant. Do they self-fertilize? Are they obligate out crossers? Do they also propagate vegetatively? Collect accordingly.

Collecting Acorns

Many non-native oaks are planted in the Sacramento Region because of their value as landscape and shade trees. Oaks are wind-pollinated and non-discriminate with their pollination; thus, they hybridize easily.* Be aware that collecting acorns from trees that have been planted as part of landscaping or are close (within 2,000 feet)** to non-native landscape oaks* have likely cross-pollinated one another producing hybrid oak acorns. **As the acorn size, shape, and cupule characteristics are determined by the mother tree, it is impossible to tell if the embryo within an acorn is true to type or a unique hybrid by looking at acorn or mother tree characteristics.** For reforestation or restoration purposes, acorns should only be collected from naturally occurring trees that are physically isolated from landscape oaks, ensuring non-hybridized oak embryos through physical separation from the presence of non-native pollen.**

1. Collect acorns directly off the tree whenever possible. Pick by hand when acorns are turning from green to rich shiny brown and come out of their caps with very slight pressure. For collection during the peak of ripening, a tarp can be spread under the tree and branches shaken with ropes or gently knocked with long poles. Be careful not to damage the tree.

2. When collecting acorns from the ground after they have fallen, look for acorns with a rich brown to slightly greenish color as they are the freshest. Different species of acorns are slightly different colors when ripe. Acorns that sit on the ground for prolonged periods of time will dry out, can overheat damaging the embryo, and their viability diminishes. As acorns dry, their color changes from glossy to a matte brown color, then a pale tan. Acorns that are very dry rattle inside their shells. Acorns that rattle will not germinate. Germination rates drop as acorns dry. Acorns on the ground may be infected by insects or have surface contamination by non-visible pathogens such as phytophthoras.

3. Choose acorns without holes or damage that are firm, not squishy. Acorns still attached to their caps are generally not viable. Make sure the inside of the acorn does not rattle when shaken. Acorns that are not uniform in color or appear wrinkly are also suspect. Though acorns can still germinate even with significant insect damage, we will try to collect only the healthiest of acorns as we intend to store them for a significant time period. Leave behind damaged and poor-quality acorns at the harvesting site as they are a vital food resource for many of our native animal and insect populations.

4. Acorns grow best when they are planted within a few days of the collection date. If acorns must be stored, close attention must be paid to temperature and humidity to ensure their continued viability. Acorns cannot be stored for more than 3-4 months.***

5. There are several techniques used for storing acorns. One is to fill a zip-lock bag no more than half full with acorns. Add enough vermiculite (bought in bags from a local nursery/hardware store) to fully cover all of the acorns. If acorns are dry when placed in the zip-lock, add 1 tablespoon of water for a gallon zip-lock bag or ½ teaspoon for a quart bag. Do not add water if acorns are damp or the bag has condensation on it. Place the bags in the refrigerator until ready to use. The goal is to keep the acorns cold and at a consistent humidity, so they do not dry out. If the vermiculite should ever look wet, remove and replace with dry vermiculite. Wet acorns will either germinate or mold. Dried out acorns cannot grow.

6. Many people like to use the "float test" to sort good acorns from bad. This is done by placing the acorns in a bucket of water. The ones that float are not good. Though helpful for sorting large quantities of acorns, the Tree Foundation only uses this test after storage before planting. If you float test the acorns before storing, you increase chances of the acorns germinating early in the fridge as well as the chance for them to mold during storage. Float tests are also an ideal way to spread pathogen contamination. If you must float test acorns, let them dry overnight before storing.

7. In locations where sudden oak death (*Phytophthora ramorum*) or other pathogen contamination is possible, acorns should be carefully cleaned before storage to minimize pathogen spread. First, make sure all acorn shells are clean and free of mud or soil. Wash them prior to treatment to remove soil if needed. Place clean acorns into a 10% bleach solution for 10 minutes. Rinse 2-3 times with clean water. Place acorns on a clean sheet or tarp (not one that has been out in the field where it could also become contaminated with pathogens) and let the acorn shells dry overnight before packaging for storage. Packing wet acorns will increase germination in storage or lead to mold.

*The general rule is that only oaks within the same section within the sub-genus can hybridize. Please see our Quercus sub-genera list or check the International Oak Society webpage to determine the possibility of hybridization between two species.

**There have only been a few studies on oak pollen movement and how far it can travel. It seems clear that it would be very dependent on local conditions including prevailing wind direction and wind speed. We have chosen the 2,000-foot separation as our basic metric but would suggest greater physical separation be considered for projects with a higher level of confidence needed that oak progeny are 100% un-hybridized.

***There is some debate over length of storage and how storage can increase or reduce viability and germination rates. Some species can be stored longer than others. The only proven way to store viable acorns over multiple seasons is in a very cold liquid nitrogen

refrigeration system. In general, we plant acorns that have been stored for 1-3 months with very high (>90%) germination rates.