

Purpose of sampling

During the 2010 field season we are implementing a pilot study that requires large-scale collection of genetic material. There are many possible uses for the data, including broad-scale mapping of genes and following the fate of certain genetic characteristics following disturbance (like the winners and losers after blister rust infection or insect infestation). Some of the most useful information may come years down the road, after we have established a baseline. Since this is a pilot, feedback from the field will be especially useful toward getting the best data possible.

Materials:

- Coin envelopes (kraft-type paper, 2 ½" x 4 ¼" or 3" x 4 ½")
- Pre-printed labels (2" x 4")
- Silica-based cat litter (like the desiccant packed with cameras and electronics, not clay-based), e.g., Litter Pearls[®], Pestell Clear Choice[®], Exquisicat[®] Crystals. **Safety note: There has been some concern that the litter may contain crystalline silica, which is a respiratory irritant. However, the products should contain only silica gel and not any crystalline silica. See brand labels for warnings, if any.**
- Gallon zip-lock freezer bags
- Ice chest (optional)

Methods

What to sample

One sample from each tree species on each subplot. For example, if you are in an aspen stand with a sparse fir understory, you might have aspen on all four subplots and fir only on two subplots. There should be six trees sampled – 4 aspen, 2 fir. Within a subplot, it is not important which tree is sampled, except that selection should follow the tissue preference list (see below). If it is not possible to sample the trees on a subplot because they are all unsuitable or material is unreachable, material may be collected off-plot (see further instructions below). It is not necessary to go through extraordinary means (e.g., climbing, shooting, throwing rocks) to obtain tissue samples. Although our goal is to sample every species on every plot, aspen and Douglas-fir are the priority species. Therefore, if circumstances limit collection the priority species should be sampled and the reason for not sampling others should be made in the crew notes.

Sample material selection

Sample quality is important, because it affects the ability to extract genetic material. Diseased, dead, or insect-infested tissue should be avoided. Tissue priority is:

- 1) Young green leaf tissue. For broadleaved species this should ~1 in. square surface area. This can be one leaf or a composite multiple small leaves on the same tree, but leaves should not be torn. For conifers, at least 5 to 10 needles per tree, depending on size. E.g., for true firs, Doug-firs, hemlocks, etc., the most recent inch or two of branch growth with all needles attached; for pines, a few fascicles with all their needles; for junipers and other scale-needled species, a few branch tips with all leaves attached.
- 2) Mature green leaf tissue. Same specifications as #1. It may be necessary to collect mature foliage during early and late-season plot visits.
- 3) Bud tissue. One to four buds per tree, depending on bud size. This will be necessary when sampling broad-leaved species during the leaf-off period, or when buds have set but leaf tissue is unsuitable (e.g., damaged, diseased, or senesced).

Sample material packing and storage

When visiting a plot, the crew should carry one or two gallon-sized zip-lock bags, partly filled with desiccant, and enough labeled coin envelopes to store the anticipated number of samples (previous plot data should give an indication of how many samples will be needed). Use the following steps to store samples:

- Sampled material should be at normal moisture levels, so if samples are being collected under rainy conditions they should be tamped dry before storage.
- Label the envelope (one per sampled tree) with the required information. If the sample is from a seedling, write “seedling” in place of the tree number. In cases where the tree was sampled off-plot, record the nearest subplot number, write “off-plot” in the tree number field, and note the reason in the label comment line (e.g., “unreachable” or “leaf disease on plot”).
- Put a few granules of desiccant inside the sampling envelope to keep it a bit open and allow air flow. Place samples in the envelope and seal the flap. For leaves or needles that are larger than the envelope size, try to fold them without tearing or breaking. If size is a problem for some species, it may be necessary to acquire larger envelopes.
- Envelopes should be sealed and then submerged in desiccant within the zip-lock bags. A 1-gallon zip-lock freezer bag should be able to contain up to about 30 sample envelopes, depending on envelope size and bulkiness of the samples. The bags should be kept as cool as possible during transportation back to camp.
- Samples should be transferred to a larger container that is filled with desiccant as soon as convenient. There are a few options here, but the important thing is that the container remains as cool and well-ventilated as is practical. The silica crystals are commonly available in a 4- to 8-pound container size (about like a 1- or 2-gallon jug) with a large screw-on lid. These containers should be able to carry a large number of sample envelopes and they can be packed conveniently for shipping. An alternative is to use an ice chest that is about ½ filled with desiccant. The lid should be left open when not being transported and the sample envelopes should be buried in the desiccant. If the samples have been handled properly, they should be storable in this condition for several weeks or more.
- Although the samples can be stored on site, the genetics laboratories will be ready for analysis any time that the samples can be provided. As a result, samples should be shipped back to Ogden when it is convenient and there are enough samples to make it worth packing. The silica material is nontoxic and non-hazardous, so ordinary ground shipping (e.g., USPS or UPS) is adequate. If the ice chest storage option is used, samples should be put into smaller containers for shipping (e.g., original litter jugs, zip-lock bags, or other sealable container). Use best judgment as to what arrangement is most likely to survive shipping!

Contact information

If there are any questions or suggestions, please email or call (any time) John Shaw (jdshaw@fs.fed.us; cell 801-598-5902).