

Collecting and Handling Protocols

General Information

What do I need to provide to the propagation facilities when I submit my samples?

1. Provide whenever possible the Rare Plant Field Data Form. If not, include with plant material sample descriptors such as:

- Genus, species, subspecies, *etc.*
- Collection organization
- Collector
- Date of collection
- Collection site
- Collection number
- Type of material
- Purpose of collection

This is to ensure accurate documentation of the plant samples.

2. Label all samples legibly and unambiguously. Make sure all samples are tagged.
3. If any special or significant sampling methods were used, note what was done.
4. Note any pest problems associated with the parent plant at the time of collection.
5. If possible, make arrangements with the propagation facility before sample collection.
6. Submit samples to the propagation facilities **as soon as possible!** Delays may have deleterious effects on sample viability.

How do I handle my plant samples after I collect them?

1. Insulate from heat. Keep at ambient to cool temperatures but do not freeze.
2. Try to cushion material so it won't be crushed.
3. Do not pack samples with excessive moisture or allow samples to sweat in the bags for an extended period of time. This promotes fungal and bacterial growth and accelerates the decline to sample quality.
4. Send to propagative facilities as soon as possible.

Collecting and Handling of Seed Propagules

Seed quality is primarily dependent upon the seed collector's methods and post harvest handling of material. Knowledge of timing and habit of natural seed dispersal is helpful (though not always available) in seed collection. Attention to inflorescence structure and their seed maturity patterns are also important in determining what to harvest.

Loss of seed viability is due to:

1. Excessive temperature.
2. Development of anaerobic conditions around the seeds caused by their own respiration. This is due to storing in plastic bags or tight packing.
3. Prolonged time interval from collection of samples to propagative facilities under conditions conducive to fungal and bacterial growth. Samples of fleshy fruit stored in plastic bags should be aerated intermittently in immediate delivery is not possible.

Dry dehiscent Only available before it disperses. Try to harvest just before dehiscing.

Dry indehiscent Dependent upon when and how dispersed. For example, wind dispersed, by animals or insects, *etc.*

Fleshy fruits Need to know if recalcitrant (desiccation intolerant) or orthodox (desiccation tolerant).

Recalcitrant Seed

Recalcitrant seeds cannot withstand any drying. Some have seed coats adapted to prevent excessive water loss while others have no such adaptation and are prone to rapid water loss post harvest.

In fleshy fruits, high seed moisture can be maintained by keeping the fruit intact. Seeds can be stored in impermeable plastic bags, but must be aerated by opening the bag intermittently to compensate for the restrictive gas exchange environment.

Insulate against heat and temperature extremes. Try to maintain a temperature as close to ambient as possible.

In mature fruit, indicate if picked off the ground or parent plant. Try not to collect from the ground if possible, unless it is known that they have recently fallen.

Orthodox Seed

In general, the desiccation tolerance of orthodox seed varies throughout its development. They tend to be intolerant of drying during early development and become more tolerant as the seeds mature.

If the fruits are immature, leave the seed within the fruit. Treat in the same manner as recalcitrant seeds.

Mature seeds from dry indehiscent or dehiscent fruits can be kept in permeable containers such as paper or cloth bags.

Collecting and Handling of Vegetative Propagules

Successful propagation of vegetative propagules is dependent upon many different factors such as the vigor of the parent, the collection date and even the environmental conditions at the time of collection. Correct handling of vegetative material is also important.

1. Vegetative materials deteriorate quickly post harvest and quick transfer from field to the propagative facility is imperative to ensure maximum viability.
2. Additional care must be taken during transport since they are easily damaged.
3. Place under cool conditions, such as a cooler with ice packs, as soon as possible after collecting and during transport to the propagation facility.
4. Try to collect samples that are insect and disease free.
5. Minimize damage during harvesting and transport.
6. In the case of vegetative cuttings, cut ends can be wrapped in damp towels or newspaper.

Vegetative Cuttings (Herbaceous)

The shoots harvested should be from the last mature flush of the plant. Cuttings should be long enough to allow for trimming and possible division.

If the plant species is known to be hard to propagate, small rooted plant suckers with some of the soil surrounding the roots could be taken if possible. Whole plants should not be removed at any time.

Vegetative Cuttings (Woody)

Propagation of mature trees is more difficult in general than their juvenile counterparts; but in many cases, juvenile forms are not available for collection. Whenever possible, the best material for propagation is the juvenile form. If only mature forms are available, material from their juvenile gradients may have a better chance of success.

Roots and Tubers

Timing of collection is important. The collection of immature or sprouting storage organs can result in significant losses in viability. In the case of plants that possess a dormant stage, a two-visit strategy may be required. One to identify individual clones and mark their location and another to collect the tubers or rhizomes once the top of the plant has died.

Fern Fronds

Fern fronds should be kept in plastic bags and not allowed to dry out during transport. If immediate delivery to the laboratory is difficult, place frond between 2 sheets of paper and allow to air dry flat within a plastic bag propped open. Spores will fall off frond as it dries. Seal the bag shut when completely dry and maintain a flat position to keep the spores on the paper surface.

Flowering Shoots

Some flowering shoots contain vegetative buds that do not develop but remain dormant. Sometimes the dormancy can be broken to produce juvenile vegetative shoots. Also, the immature flowers of a few tree species have been known to form adventitious shoots.

Root Cuttings

When lateral shoots are not available, such as in palms and other monocots, it is sometimes possible to produce vegetative shoots from root cuttings. Roots are often considered to be more juvenile in age than most of the tree. A juvenile gradient exists for roots, with the most juvenile material being closest to the trunk. Sprouts arising naturally from the roots of trees generally are juvenile in form. Store root cuttings in a moist sterile medium, such as peat moss.

Decontamination of Collecting Tools

Many of the Hawaiian endemic species have limited or non-existing *ex situ* collections, which necessitates the need for active *in situ* collecting. It is imperative that precautions be taken to keep the natural populations as disease free as possible. This is not only to maintain clean propagative stock material during collections, but also to ensure the integrity and overall health of the existing population and the surrounding flora. While absolute elimination of all pathogens is impractical and impossible, procedures should be directed toward preventing the introduction of serious foreign pathogens.

The risk of disease transmission of viral, fungal, or bacterial origin is a realistic possibility through the cutting implements used in collection of plant samples. Whenever possible, plant cuttings should be made with a new, unused blade. This can be accomplished by using an implement such as a box knife fitted with a disposable razor blade. The used blade can be changed before cutting the next sample.

Dr. Stephen Ferreira at UH Plant Pathology has also suggested that any cutting of plant propagules performed post collection should be done with disinfected tools. This is to prevent any disease contamination of the propagules before it goes to the propagation facility.

Decontaminate tools:

Make a 5 % to 10% solution of household bleach (such as Clorox manufactured by The Clorox Co.) and soak tools. Let sit for 2-3 minutes then rinse well with water. Always use a fresh batch of bleach solution.

References:

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Falk, D.A. and K.E. Holsinger (1991) *Genetics and Conservation of Rare Plants*. Oxford University Press, Oxford, New York.

Guarino, L., V. Ramanatha Rao and R. Reid (1995) *Collecting Plant Genetic Diversity-Technical Guidelines*. CAB International, Oxon, UK.

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