Propagule Collection and Handling

Collecting propagules for propagation or storage is a common conservation action for rare plants in Hawai'i. Propagules are frequently submitted to nurseries, seed banks, botanical gardens and the Lyon Micropropagation Lab for immediate propagation or long-term storage. Following best practices in both the collecting and post-harvest handling stages of propagule collection is essential to providing *ex situ* collections that will be healthy, maintain good quality, and store or propagate well. These practices will also protect wild populations from over-collection and maximize their representation *ex situ*.

Sampling a Population

The Center for Plant Conservation (CPC) Best Plant Conservation Pactices provides an excellent overview and updated protocols and are adopted in full by the HRPRG: https:/saveplants.org/best-practices/why-conserve-rare-plants/). Some essential guidelines and best practices are summarized here:

- Prepare in advance with permits, clean equipment, and sampling strategy; time visits with phenology
- Communicate with other collectors to avoid duplicating efforts and over-collection
- Notify *ex situ* facilities in advance that a collection will be submitted so they can prepare
- Capture diversity by sampling across the spatial expanse and morphological diversity of each site
- Sample across multiple weeks or seasons whenever possible to capture the range in phenology
- Capture at least five subpopulation units from across its distribution in each ecoregion
- Collect and maintain separate accessions from up to 50 maternal plants from each subpopulation
- Collect no more than 10% of an individual plant or population's seed production in one season
- Collections of vegetative material should be limited to 10% or less of the material available on each plant; or, no more than six cuttings, whichever is less. Cuttings should be a maximum of 8-inches long, or have just two nodes if longer. Only apparently healthy plants should be collected.
- Extremely small populations or populations with high probability of extirpation in the foreseeable future may warrant rescue collections greater than 10%. For populations that have 10 or fewer reproductive individuals and a poor history of recruitment, or are known to be in precipitous decline, it may be necessary to rescue the population from whatever is threatening it in the wild population by collecting up to 100% of seed. This decision is left to the discretion of the permitting agency.

Bulk vs. Individual

When collecting propagules - such as seeds or cuttings - from wild plants for eventual outplanting, one major decision is whether to use propagules from separate individuals (INDIVIDUAL) or combine them (BULK). Propagules can be labeled and managed separately or be combined at any of the steps in the process: when collected, packaged, processed, tested, rooted/germinated, kept in the nursery, or outplanted. It may be cheaper or simpler to combine them, but from that point on the plants cannot be tracked back to their founder. Collections of Threatened and Endangered species, Plant Extinction Prevention Program (PEPP), or other rare plants should always be from individual tagged plants and kept separately, so managers can properly manage any diversity in the remaining founders to design outplantings. Even if collections from each individual plant are not kept separate, collections from different populations should be.

When to keep collections by individual plant:

- PEPP or ESA-listed (Threatened/Endangered) species
- Research (e.g., taxonomy, seed biology, ecology, fitness)
- Collecting from fewer than 10 plants per population

Collecting by individual can be useful, for example, when 1) taxonomy is unclear; 2) differences in storage behavior between founders is suspected; 3) measuring variation in germination or growth rates of seedlings from unique founders; 4) determining the most suitable founders for use in a restoration site; 5) amplifying seeds in an orchard setting; and 6) when there are resources to tag, track, and analyze the data through the propagation and outplanting. While this takes more time and resources, it is the only way to monitor for success and is the only approved collection method for rare, threatened, and endangered plants.

Collecting in bulk can be useful for non-rare species, for example, when 1) species are common (not rare); 2) taxonomy is clear; 3) founders cannot or will not be tagged; 4) suitability for restoration is already known (so will not be monitored); 5) for use in seed sowing projects; and 6) there are insufficient resources to tag, track, and analyze the data throughout the process. This is often cheaper, quicker, simpler, and requires less training.

Labeling a Collection

The HRPRG Rare Plant Monitoring Form and a purpose (immediate propagation, research, short/long-term storage, etc.) should be communicated to *ex situ* facilities over email within 48 hours of the submission. Label all samples legibly and unambiguously. If any special or significant sampling methods were used, note what was done. Note any pest problems associated with the parent plant at the time of collection. At a minimum, the bag or envelope with each collection must be physically labeled in permanent marker with the following information:

- Genus, species, subspecies/variety. Call if it is a new taxon or are unsure about what it is.
- Collector Name
- Date of collection
- HRPRG Geographical Reference Code and individual plant number (see Naming Rare Plant Locations BMP; ex. OA-MAK-A-00001)

Collection Handling

Collections must be handled carefully while being transferred for propagation. With rare plants, initial quality of collections is often out of the collector's control. However, using good post-harvest handling techniques can make a major difference in the ultimate quality and viability of a collection. Here are a few basic best practices:

- Submit samples to propagation facilities immediately. Delays have deleterious effects on viability.
- Insulate from heat, and aim to keep the collection at the same temperature as the collection site
- Cushion and protect the material so it won't be crushed, bent, or broken
- Do not pack samples with excessive moisture or allow samples to sweat in the bags. This promotes fungal and bacterial growth and accelerates the decline of sample quality.

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.

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/intermediate (desiccation and low temperature tolerant).

After harvest loss of seed viability is primarily due to:

- Excessive temperature during transport
- Development of anaerobic conditions around the fruits caused by their own respiration, which also increases temperature. This is due to storing in plastic bags or tight packing.
- Prolonged time interval from collection of samples to delivery to propagative facilities, creating conditions conducive to fungal and bacterial growth.

To avoid these problems:

- Keep seeds/fruits at the appropriate temperature. For any fleshy fruit that is not recalcitrant, if they cannot be delivered immediately, keep them in the refrigerator (check with the seed laboratory if you are unsure whether they are recalcitrant). See the Hawai'i Seed Bank Partnership User's Guide for more information about which species are recalcitrant: http://laukahi.org/hawai'i-seed-bank-users-guide/
- Avoid storing fruits in sealed (air-tight) plastic bags. Dry fruits can be stored in paper packets or envelopes, and fleshy fruits can be stored in mesh bags, such as organza bags or tea bags, all of which are breathable. If using plastic bags in the field, it is best to transfer them to appropriate containers as soon as you can for any temporary storage, especially shipping.
- If you must collect a fruit that is already beginning to ferment and cannot deliver it immediately, ship material ASAP. If needed, place mesh bags in open plastic bags to avoid compromising the shipping box. If you have no other option, process the fruit and let the seeds air dry before shipping. Call your seed bank for guidance on how to process.
- Deliver seeds/fruits as soon as possible. If mailing, use the appropriate containers listed above. Make sure the collection will not arrive on a Friday afternoon, or especially a Saturday, sitting over a weekend is often very detrimental to seeds. If you cannot mail collections before Thursday morning, you are better off refrigerating non-recalcitrant collections and sending first thing Monday morning. Always give the facility a heads up when shipping.

Recalcitrant Seeds

- Recalcitrant seeds cannot withstand any drying or possibly any temperatures below ambient. Some have seed coats adapted to prevent water loss while others do not and are prone to rapid water loss post-harvest.

- In fleshy fruits, high seed moisture can be maintained by keeping the fruit intact. Their individual seeds can be stored in impermeable plastic bags but must be aerated by opening the bag intermittently to allow for gas exchange.
- 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. If the taxon is not especially rare, try not to collect from the ground if possible, unless it is known that they have recently fallen. If you take a mixed collection, keep the fruits from the plant and from the ground in separate batches, since there may be a difference in quality, to avoid compromising the better batch.

Orthodox and Intermediate Seeds

- The desiccation tolerance of orthodox seeds varies throughout their 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 seeds 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.

- Mature fleshy fruits can be kept at ambient temperature in permeable containers, such as organza bags. If fruits need to be in sealed containers at ambient temperature, such as when hiking, frequently open the container to allow for air exchange (ideally every couple hours). This prevents rot or accelerated aging (ethylene build up). Fleshy fruits that are not processed immediately can be stored in sealed containers and refrigerated for up to a couple days. Do not store fleshy fruits in open containers that would cause the fruit to dry.

Post-Harvest Ripening

If you have no other option than to collect immature fruits, *sometimes* they can be post-harvest ripened (contact facility to find out if possible). Collect a good amount of vegetative material attached to the fruit/infructescence and treat the material like a vegetative cutting.

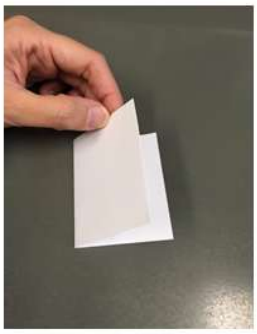
Paper Envelopes for Dry Fruit/Seeds

Envelopes and paper bags can often be an issue, especially for small seeds. They can have open corners that let small seeds escape or flaps that trap seeds. If you need to use envelopes, consider placing the seeds in a coffee filter first and then into an envelop) OR make sure the corners are closed, and **do not** seal using an adhesive strip (which can ruin smaller seeds if they get stuck to it), but use tape on the outside instead.

We also recommend a more effective and cheaper alternative for small seeds or spores: the paper packet. It is simple and quick and will not waste any seeds. For temporary storage (shipping), you can use regular computer paper, even scratch paper as long as labeling is clear. This option is also cheaper than envelopes. Then several packets snugly placed into one larger sealed envelope gives an extra layer of protection. See the visual guides below.

Visual Guide for Seed/Fruit Post-Harvest Storage. How to make paper packets:

Start with a sheet of paper or ½, ¼, ⅛ sheet)

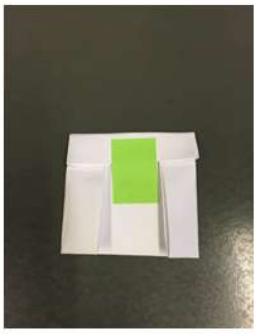


Fold it in half

Fold in the two parallel open sides

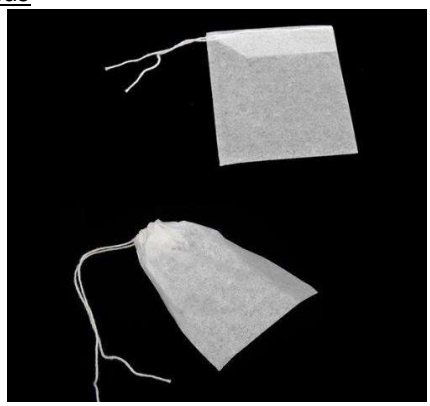
Fold in the remaining open end, towards the other folds

Open the top flap to fill the packet with seeds



One secure piece of tape will prevent spillage

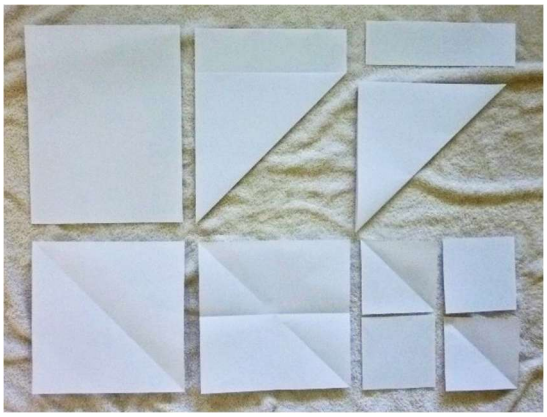
Mesh Bags for Fleshy Fruits and Wet Seeds

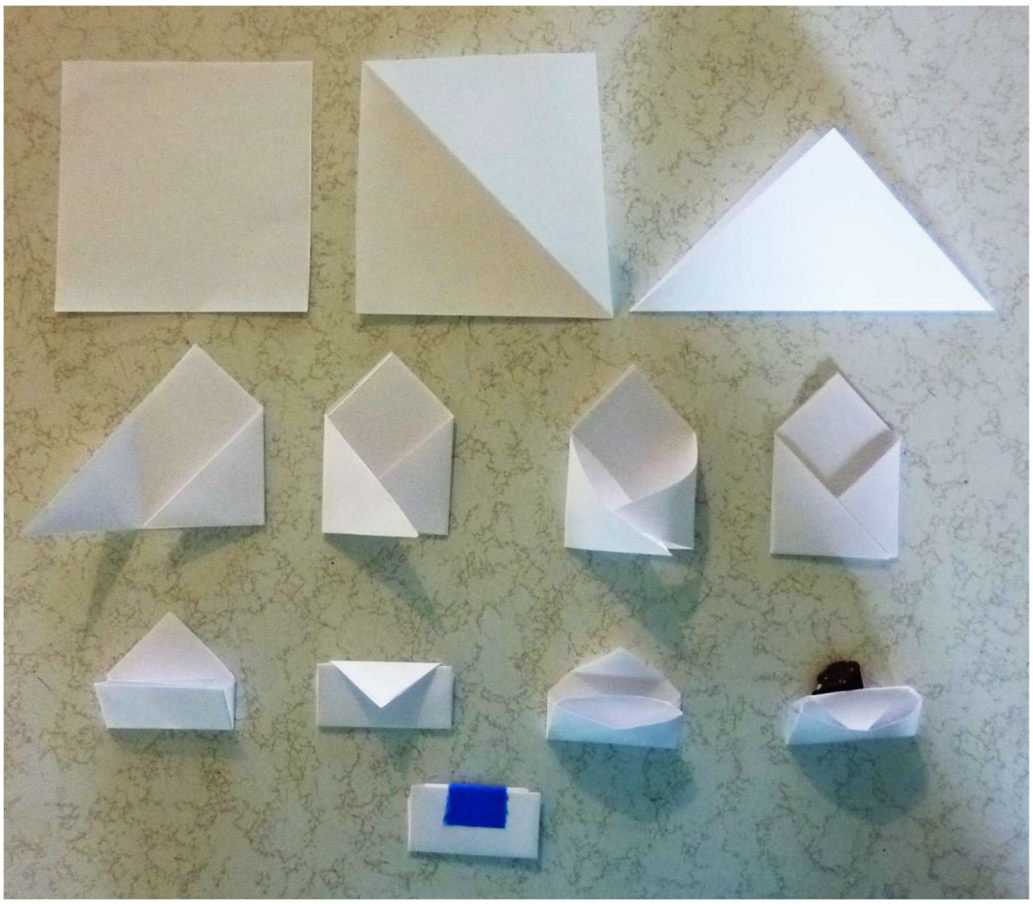


Organza bags and tea bags (for loose tea) work well for fleshy fruits. Just include a label with the information above. They also work well for bagging fruits on plants in the field.

Maui Nui Botanical Garden ʻōhiʻa seed packets (courtesy of Cathy Davenport):

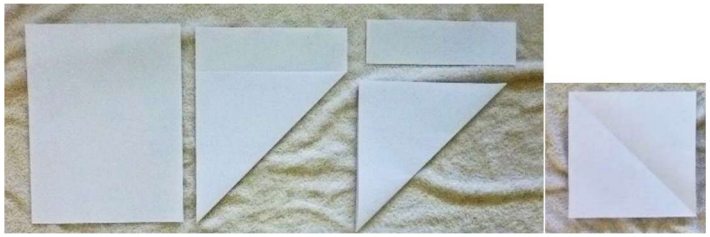
Seed Storage (small packets). ʻŌhiʻa seed small packages. Used for storing the 100 count seeds in the foil packets. These don't need to be double-folded edges, like the field packets. These will not be jostled around in the field. Start with a small square. There are four small squares per 8-1/2"x11" paper. Before putting the seed into the package, label the package (handwritten). Use pencil only. (No pen please.)

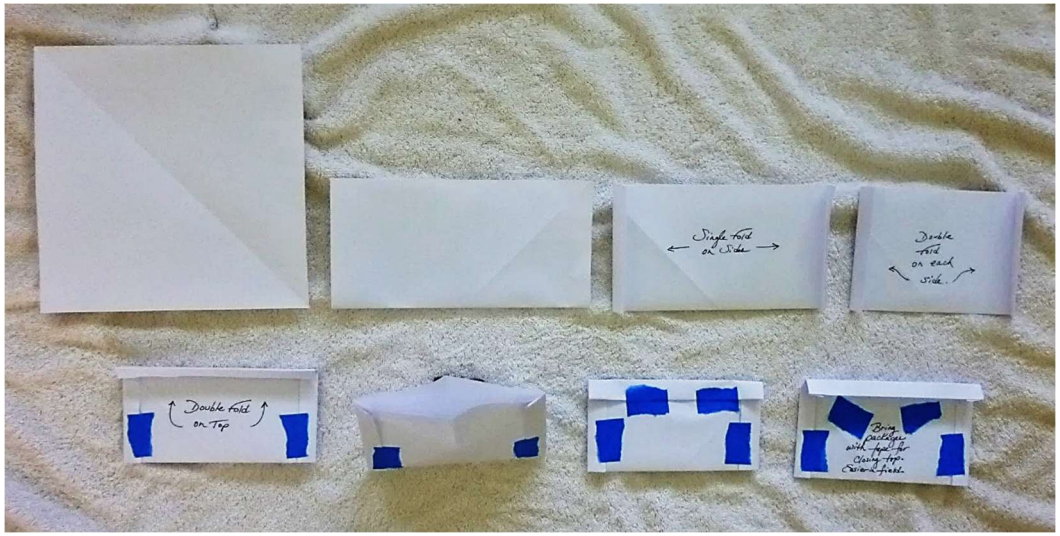




Field (Large) Collecting Packets. For seed collection in the field.

Using a coffee filter is best for collecting small seed, especially with small seed like ʻŌhiʻa. The seeds are out of the wind while collecting. After collecting seeds, roll up the filter edges, fold the ends inward. Then place filter into coin envelope. No need for tape, which the seeds often find their way to, and stick to. No need for tape leaves one less thing to bring. If no coffee filter, double fold the edges. Otherwise the seeds find their way out and some stick to the tape. (Be sure to include the tape for closing the package in the field.)

Start with 8-1/2”x11” paper. Make a square. 



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. Proper handling of vegetative material is also critically important. Here are a few basic best practices:

- Vegetative materials deteriorate quickly post-harvest and quick transfer from field to the propagative facility is imperative to ensure maximum viability.
- Collections should be kept in sealed containers and/or plastic bags to retain moisture and protect the material during transport
- Additional care must be taken during transport since they are easily damaged
- Place under cool conditions, such as a cooler with ice packs, as soon as possible after collecting and during transport to the propagation facility. Do not allow material to come into direct contact with ice.
- Try to collect samples that are insect and disease free
- Minimize damage during harvesting and transport
- Wrap cut ends in damp towels or newspaper. Keep from drying out or wilting.

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 (see figure below).

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.

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.

Fern Spores

Unlike flowering plants, ferns reproduce by means of spores. These spores are very small and can be hard to see with the naked eye unless in large quantities. Making a successful collection of spores takes some practice and a little luck. Most important factor to consider when attempting spore collection is maturity of spores. Most fern species tend to develop and release spores on a seasonal basis, so timing of collection is a critical factor. Mature spores and sporangia will often be dark in color and uniform in texture, although it is best to look for spore maturity characteristics on a species-by-species basis. This is because fern spores can range in color from green to black, or even yellow. A magnifying device can be utilized to determine spore maturity and texture of sori (small clusters of sporangia formed together) and sporangia (globular structures holding the spores). If these structures feel or look as if they have a rough texture, it is likely that the annuli are uncoiled and the spores have already been released. For many species sporangia and spores are covered with indusium (thin layer of frond tissue covering the developing sorus) will open (brake) close to optimal spore maturity. This can be used as indicator for proper collection timing.

Collection of vegetative material from ferns should be done conservatively because plants can be easily damaged if over-collected. One fertile frond can yield hundreds of thousands of spores, so large amounts of material need not be collected for successful propagation. Thus 1 - 3 pinnae or small section of the frond would be enough to be sampled for propagation. Collected material should be placed in a folded paper in a bigger paper bag, until taken to the lab. To reduce the contamination with the spores of other species, fern fronds could be rinsed with water to wash off any unwanted fern spores. After spraying down with water, green spores should be placed in a sealed plastic bag to maintain a moist environment until material can be processed in laboratory setting. Maintaining a moist environment is crucial in case of green chlorophyllous spores, which may lose their viability within few days. Collected material should be kept in cool (refrigerator in the lab, thermos-box in the field), however, the quicker the collected materials can be processed, the better.

Processing of collected fern material should be done in an environment with as few contaminants as possible. Microscopic spores, from ferns and mold, can be floating in the air all around you, which can lead to contamination. Collected material should be placed in a folded paper packet and kept in dry conditions for 24 hours. Within this time the frond or pinnae will dry out, and this will in turn trigger the annuli to uncoil and release spores. After approximately 24 hours of drying, all mature spores should be released from the collected material. Clean paint brush can be used to brush the spores from the surface of the paper packet. Most likely a combination of spores, sporangia, and other vegetative debris will be a resulting product. This can then be sifted with a #230 (63 micron) sieve to achieve pure spores. Examples of three stages of fern spore maturity of Christella sp.

Examples of three stages of fern spore maturity of *Christella* sp.

Figure 1 is an example of immature sori which are covered with pale green or whitish indusia. The spores in there are still in developing stage and it may take a week or more, depending on the species, and you might have a better chance of finding ripe spores.

Figure 2 is an example of sori and sporangia that have already released their spores. Note the coarse texture and light brown color. This material is far past optimal collecting condition. Collection of this quality of material will result mostly in debris of sporangia and very few spores, you are better off waiting until next season for more mature material.

Figure 3 is an example of a fern that has just reached optimal spore maturity. Some of the sporangia near the tip of the pinnae have already begun to release spores, but the majority of the sporangia are at the right stage of maturity. Note the dark brown/black color of the spores and smooth texture of the sporangia. Spores normally ripen in sequence from the base to the tip of the frond, therefore ripe and unripe spores can be found on the same frond. Upon collecting and processing several pinnae, this fern yielded several tens of thousands of spores ready for propagation.

Sanitation

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. It is imperative that precautions be taken to ensure the integrity and overall health of the existing population and the surrounding flora as well as to maintain clean propagation stock material during collections. While absolute elimination of all pathogens is impossible, sanitation protocols that minimize the risk of introducing serious foreign pathogens should be mandatory procedural requirements in any collection activity from wild plants.

Whenever possible, any plant samplings that require cutting into or off a wild donor plant 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. If the use of disposable blades is unsuitable or unavailable, decontamination of your cuttings tools (shears, pruners, knives) can still be accomplished through soaks in various cleaning products. Read the manufacturer's label, material safety data sheet and application recommendations for proper usage and disposal. The disinfection treatment soaks of tools require some time in solution, so it is best to work with several pairs at a time. This way, you can have one to work with immediately and another soaking.

The described disinfection treatments can significantly shorten the life of your tools. To prolong the life of your cutting tools, rinse in clean water, dry, and oil (e.g. Felco 980 spray) the blades at the end of each work period.

Some suggested disinfectants are:

1. Chlorine Bleach solution: Make a 5–10% solution of household bleach. Rinse tools with water to remove any visible debris or plant sap. Soak rinsed tools in bleach solution for 5–10 minutes then rinse well with water. Do not store pre-made solutions, always use a fresh batch of bleach solution made the day you do your cuttings. Warning: Chlorine bleach is very corrosive to your tools.
2. ZeroTol™: ZeroTol is a broad-spectrum algaecide/fungicide formulated to kill active and dormant spores on contact. ZeroTol is plant-safe, and can be used on tools, pots, trays, structures, concrete walkways, gravel/dirt walkways, stock tanks, and irrigation lines. For disinfestation of cutting tools, use a dilution of 1:300 or ½ fl. oz. per gallon of clean water. Immerse cutting edges of tools for at least 10 minutes. ZeroTol has a minimal amount of surfactant added in their formulation to improve the wetting action of the solution, but additional surfactant may be added if needed. Do not dry off and use the wet tool to make your cuttings. Repeat the disinfection soak after use on each plant to kill and prevent transmission of plant disease organisms.
3. Consan Triple Action 20™: Is a disinfectant, fungicide, microbicide/microbistat, sanitizer, virucide and algaecide for birdbaths, greenhouses, cutting tools, flowerpots, and algae covered hard surfaces. Soak the cutting edges of plant tools in a solution of one teaspoon of Consan to one gallon of water for at least 10 minutes. Do not dry off and use the wet tool to make your cuttings. Repeat the disinfection soak after use on each plant to kill and prevent transmission of plant disease organisms.
4. Miscellaneous Biocides: Some regular commercially available household cleaners might be useful as disinfectants for cutting tools or hard surfaces. Read the manufacturer's label to see if the product is suitable for your specific needs. For example, Proforce from Ecolabs, sold at Costco Wholesale, a one step disinfectant, cleaner, sanitizer, fungicide, mildewstat, virucide, and deodorizer, with a recommended rate of 1 oz per gallon of water.
5. Flaming tools: An 85% ethyl alcohol dip followed by 6–8 seconds flaming with a mini torch or Bunsen burner is sometimes used to clean cutting implements. It is not recommended nor employed very often in field work due to the hazardous nature of this procedure. This method is suited more for an environmental setting where the risk of starting a fire or causing injury by fire is controlled and minimized, such as in the greenhouse or propagation facility.

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Schmidt, L. (2000) Guide to Handling of Tropical and Subtropical Forest Seed. Danida Forest Seed Centre: Humlebaek, Denmark.

Online Resources:

Kew Millenium Seed Bank Resources (including post-harvest handling protocols)
http://www.kew.org/science-conservation/research-data/resources/millennium-seed-bank-resources

ENSCONET Collecting Protocol
http://www.kew.org/sites/default/files/ENSCONET_Collecting_protocol_English.pdf

Seeds of Success (BLM) Collection Protocols
http://www.blm.gov/wo/st/en/prog/more/fish__wildlife_and/plants/seeds_of_success/protocol.html

HRPRG Geographic Reference Areas:
https://histategis.maps.arcgis.com/apps/webappviewer/index.html?id=de12e4e512824f90b8be4fa68ce9e9c1

HRPRG Best Management Practices: https://laukahi.org/hawaii-rare-plant-restoration-group/

Center for Plant Conservation Nest Plant Conservation Practices: https:/saveplants.org/best-practices/why-conserve-rare-plants/