GENERAL SEED COLLECTION GUIDELINES
For California Native Plant Species

The quality of the programs at Rancho Santa Ana Botanic Garden are intimately tied to the quality of the Garden's collections. For the Seed Program, high quality collections contain seeds that germinate more reliably, produce seedlings with greater vigor, and maintain viability longer in storage. High quality seed collections provide material for collection display, research, and rare plant reintroduction and population enhancement programs at RSA and around the globe.

MAKING HIGH QUALITY SEED COLLECTIONS

Consideration of the following five questions can help guide the collector in obtaining the highest quality and therefore the most useful seed collections.

Why – defining the purpose and use of the seed collection
- Business
- Horticulture
- Research
- Restoration
- Conservation

What – defining high quality seed collections
- Correct target species identification and verification
- Healthy, sound, viable seed
- Sufficient sized collection to meet the intended uses
- Genetically representative of the species, or population sampled
- Adequate associated data to meet intended uses

Where – being at the right place
- Species distribution
- Local abundance
- Provenance
- Accessibility

When – at the right time
- Plant type
- Fruit type
- Climate
- Elevation
- Micro-habitats

How – making high quality collections
- Impact and ethics
- Sampling methods and techniques
- Collection methods
- Post harvest care of collections

Why – defining the purpose and use of the seed collection

The purpose and use of the collection, more than any other consideration, directly affects all other aspects of seed collecting. For instance, the purpose of the seed collection influences how many plants will be collected from and the sampling strategy taken. Potential use of the collection will affect the quantity of seeds that is needed, how many and which populations will be collected from. Is the seed intended for propagation, for seed banking and distribution or both? Under some situations immature seed or very limited quantities of seed may be acceptable. If larger quantities of seed than are needed are collected does one have the facilities to safely store the additional material? What is the storage tolerance of the species? Will the extra material maintain viability in storage until needed?

What – defining high quality seed collections

Quality should always trump quantity. Making high quality seed collections is the primary goal for any seed collector. It is always better to spend more time at each site in the field making fewer good seed collections than to hastily grab collections in an effort to gather a greater quantity of not-so-useful collections.

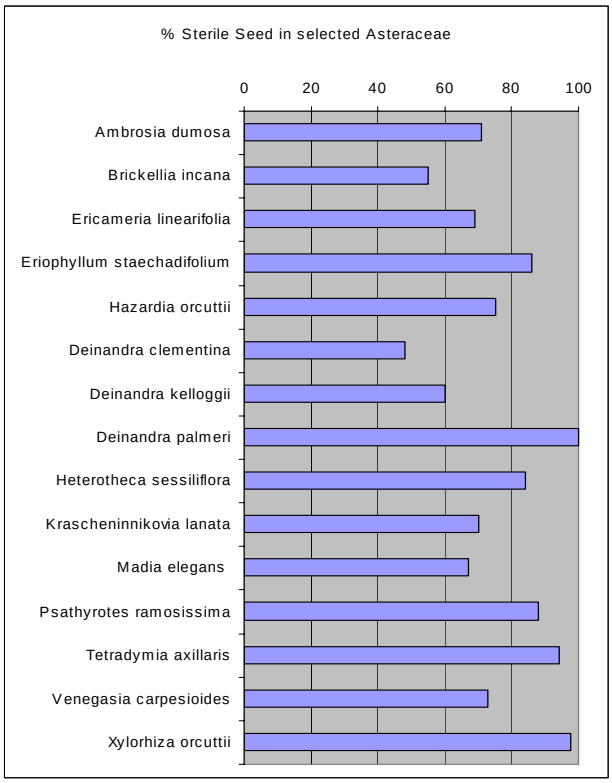
Correct species identification and making voucher herbarium specimens
The American Heritage Dictionary defines voucher as a verb “to substantiate or authenticate with evidence”. Properly collected and documented herbarium specimens provide the evidence necessary to verify a species identity. Vouchers also serve to document morphological variation and to provide historical documentation as to a species occurrence, range and distribution. For a detailed explanation on correct techniques for collecting and documenting herbarium and voucher specimens see *T. Ross, Crossosoma 22 (1), 1996, pp. 3-39.*

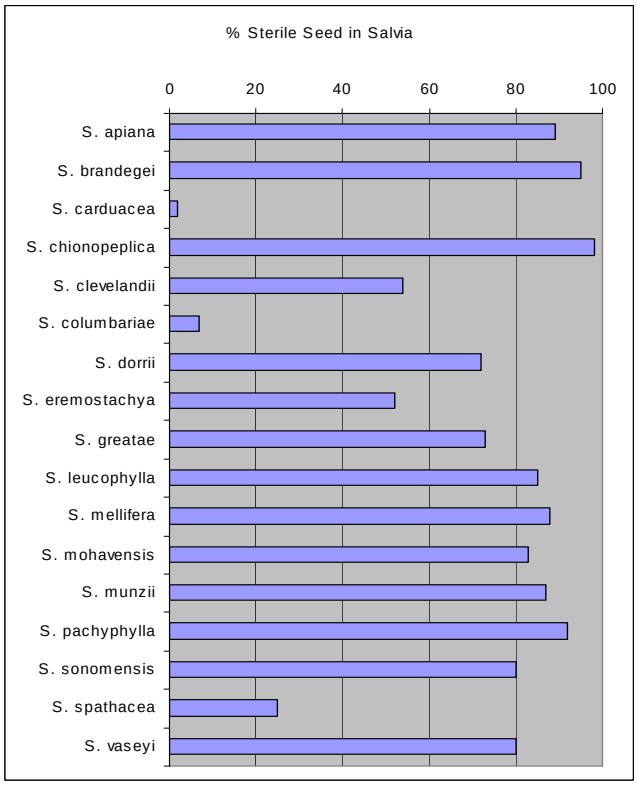
A voucher specimen is an essential component of a high quality seed collection. Typically voucher specimens are collected on scouting trips during the flowering season followed by a return trip or sometimes multiple trips to a site when fruits and seeds have ripened. If flowering voucher specimens are not made prior to making the seed collection, specimens can be made from fruiting samples. Voucher specimens should never be made if doing so will adversely affect the plant population. Good field notes, leaf or flower samples, and photographs can also serve to identify and document the existence of a plant species.

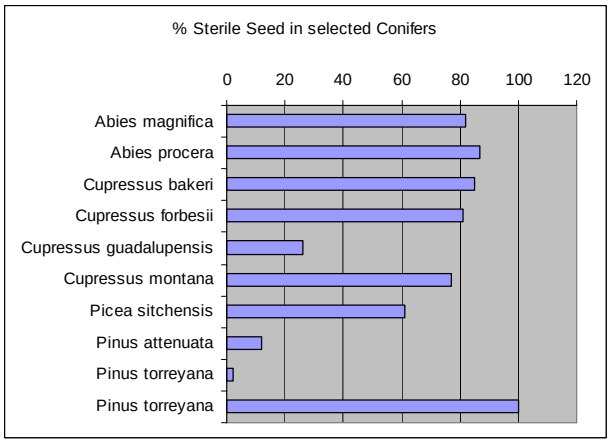
Healthy, sound, viable seed
Maximum germination, seedling vigor, and longest storage life is achieved when fully ripened, current season, pest-free seed is harvested. Environmental conditions, lack of pollinators, and parasitism all can affect the quality of the seeds collected. It is important to note in the field whether the seeds that are being collected have viable embryos. Healthy sound seed has an interior area that is filled from edge to edge with generally white and fairly moist endosperm and/or embryo tissue. Many plant species produce "parthenocarpic" or asexually produced and therefore sterile seeds. Noting the percent viability of a given seed lot allows for better seed collections and control of results during propagation.

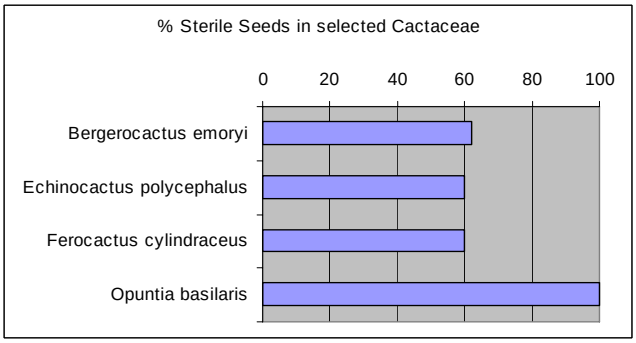
The graphs on this page represent seed lots of RSA collections where seed parthenocarpy has been documented. Percent viable seed varies from one seed lot to the next, among families and genera. Plants that produce small seeds, and most annual plant species, tend to produce a higher percentage of fertile seeds.

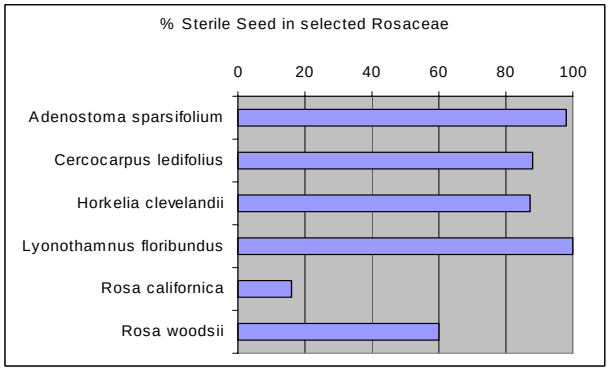
Seed Parthenocarpy

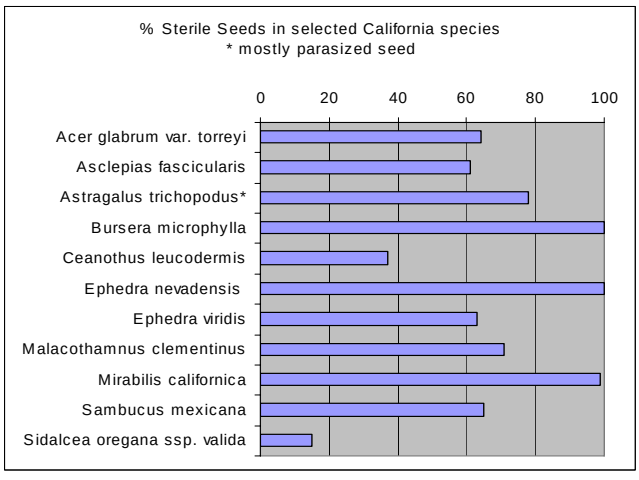












Viability assessments and considerations in the field

Two of the most common methods for estimating viability and seed quality are floatation and dissection. Making a "cut test" dissection exam to check seed development and soundness can easily be done in the field using a single edge razor, a small block of wood, a hand lens and some double-sided tape (to hold the seeds steady and to keep them from blowing away). For plant species that produce relatively large and smooth seeds (greater than 3.0 mm) a quick and easy way to check viability is to conduct a floatation assessment. Due to the presence of air pockets where healthy embryo and endosperm would normally be, hollow or partially hollow, underdeveloped, or parasitized seed will float, while filled, sound seed will sink. There are exceptions where filled and sound seed will float but a quick cut test on any floating seeds will give one a good estimate regarding the viability of the current season's seed crop.

Fertile ripe achenes easily detach from the floral receptacle while sterile and parasitized achenes will remain within the floral involucral cup long after the healthy seed has dispersed. Seed cones containing fertile seed will generally open naturally while cones with a high percent of sterile seed may not open at all. In some conifer species sterile seeds are lighter in color than the fertile ones. The quantity of fertile seed produced by a plant population and individual plants can vary considerably from year to year.

Sufficient quantity of seed

What is the purpose of the collection? How many seeds are necessary? It makes little sense to take more than one needs; however, it makes even less sense to not get enough if the material is available and abundant.

Most seed collections at RSA serve three primary uses.

1. Propagation: to support growth and maintenance of living collections at RSA and other botanical institutions
2. Conservation: to enhance the long-term survival prospects of sampled populations and species in their native habitats
3. Research: to support botanic, horticulture, and seed storage research programs at RSA and other institutions

RSA Seed Bank collection guidelines suggest a minimum of 2,500 seeds per accession as a useful "minimum" target quantity. This amount of seed allows for some immediate use (propagation and germination trialing), initial and follow-up viability testing, distribution of samples for research and horticultural use, and for some anticipated loss in viability over time. Obviously in some cases a target quantity of 2,500 seeds is not always appropriate. For example, for very large seeds, recalcitrant seeds (those that do not tolerate dessication and cold storage), and if removing this quantity could potentially jeopardize the long-term viability of the population. In some cases larger quantities of seed are warranted and appropriate. For their "Seeds of Success" program, The Royal Botanic Garden Kew targets 10,000 – 20,000 seeds per taxon. This higher quantity of material is mostly to allow for periodic sampling over an estimated period of more than 200 years.

The quantity of seed collected will be determined by many factors, including:

1. The size, rarity and biological health of the plant population
2. Collection timing, inherent dormancy mechanisms and germination response of the species
3. The quantity and quality of seeds that each plant produces in any given year.

If a species has good storage tolerance it makes good sense to collect larger quantities during banner years when seed is abundant.

Adequate associated data to meet the intended use
A seed collection with limited associated data has limited value. While in the field record the following information:

1. Collection date
2. Species identity (as can be determined in the field)
3. Descriptive and precise locality information including habitat and associated species
4. Site conditions including sun / shade exposure, soil type, aspect, degree of slope, and elevation
5. Local abundance, population size and number of individuals sampled
6. Fruiting stage of the plants sampled, e.g. early, ripe, or late

Always submit complete record information for each collection from a site. Personal field observations and a statement of the purpose for making the collection will be helpful for those handling the seed and growing plants out at a future time. A collector's voucher number or reference to an existing voucher should accompany all seed collections. Vouchering seed collections from cultivated plants and securing vouchers from collections obtained from third parties is good practice and adds to the value of the collection.

Where - being at the right place

Species distribution
Many plant species are widespread in their distribution, abundant and dependable in their occurrence. A majority of the species in California however are annuals, geophytes (plant species developing from an underground storage organ such as bulbs, corms and tubers) or rare species, famously unreliable in their distribution and occurrence. Regional floras, vegetation classification texts and maps, herbarium specimens are good resources for identifying potential collection sites. Knowing a species typical habitat, ecology, geographical distribution and associated species aid in locating populations and collection sites.

Source
The more local the source of the seed, the more likely the plants generated will be genetically adapted to local environmental, edaphic, and biological conditions.

Local abundance
Quantity and timing of rainfall, light and temperature, competition from other plant species all affect seed production. Pollinator visitation or the timing of pollen transfer can have a positive or a negative influence on seed set within a given plant population. Fire, floods, landslides and other natural disturbance, and even unnatural disturbance such as grading for a road, firebreak or development projects, followed by a good rainfall will frequently produce an abundance of plants to fill these newly created open spaces. Even the falling of one large tree can provide an opportunity for new plants to grow and increase the local diversity at a site.

Provenance
Where the seed comes from or the source of the material is an important consideration when making a collection. At RSA we track three basic collection provenance types:
1. W collected directly from the wild; origin known
2. Z from a cultivated plant of known wild origin
3. G from a cultivated plant not of known wild origin

Cultivated plants have a greater probability of being genetically "untrue to type"; in other words, different from their wild relatives. While a 'Z' or 'G' provenance plant selection may be a perfectly wonderful specimen for the garden, and perfectly appropriate for some restoration and landscaping purposes, selection pressures during cultivation can genetically compromise a plant's ability to survive on their own under natural conditions. When regenerating seed for conservation and restoration collections the source seed should be from plants of documented known wild origin and from as many unique maternal lines (parents from the wild population) as possible. Collecting seeds from plants in cultivation should be avoided when closely related species are in the vicinity. Hybridization between closely related species in the wild is also not uncommon. When closely related species are observed in the proximity of the target species this information should be noted with the collection data.

Again the use of the seed collection influences the appropriate provenance of a seed collection. Today, with an ever-increasing use of native plants in highway right-of-ways, restoration, and urban horticulture, it is becoming increasingly difficult to confidently identify a wild source plant from one with cultivated origins.

Accessibility and permits
Before going into the field, research the areas to be visited to determine if the land is private or public and, if it is public, who the land management authority is. Make certain that appropriate permits and any other required permissions to collect have been granted and are accessible while in the field.

Currently RSA maintains collection permits that grant permission for the Garden to collect on lands under the jurisdiction of the Bureau of Land Management and for many national forest regions. Some of the northern California national forests only issue permits on a case-by-case basis and contact with the appropriate forest botanist prior to making a trip is required to secure permission to make collections. The Garden also maintains a Memorandum of Understanding with the State of California and a U.S. Fish and Wildlife Service permit that allows for and regulates conditions for sampling and documenting the occurrence of state and federally listed threatened and endangered plant species. The Table in the appendices lists the Garden collecting permits contact information.

Before going into the field carefully read the terms, conditions, and restrictions stated in the collecting permit. Many permitting agencies have varying requirements and it is important that collectors are aware of and in compliance with any limitations and conditions established by the permitting agency.

While not required by all agencies, filing an annual collection report is a reasonable courtesy and fosters a cooperative and mutually beneficial relationship with the permitting agencies. Collection data, species occurrence documentation, along with observations made in the field can be of great value to land management agency personnel.

RSA annual collection reports are generated in January of each year from the Herbarium and Horticulture database systems and are sent to national forest resource managers and to the state BLM office in Sacramento. Rare plant collection reports are sent to the Department of Fish and Game state botanist in Sacramento and to the appropriate regional Fish and Wildlife Service field office supervisors.

Rancho Santa Ana Botanic Garden also maintains a CITES (Conservation on International Trade in Endangered Species) Certificate of Scientific Exchange. This permit allows for the exchange of collections of CITES listed species with other institutions that also are approved to maintain a Certificate of Scientific Exchange permit.

When - at the right time

Simply stated.

Seeds are ripe when they shake in the pod, are easily removed from the plant, and/or are turning dark in color.

Timing is critical for making high quality seed collections. The following factors influence when a species seed is ripe and ready for harvesting. Finding the target plant at the right time is part art, part science, and a bit of luck. Field notes from previous seed collections, floras, herbarium specimens, and of course one's own or other's experience are all useful sources of information.

Plant type
In general, annuals and perennials will be ready for seed collection 2-5 weeks after peak bloom while shrubs and trees may take two months or longer for fruits and seed to mature. A few plant species produce fruits that require two seasons to fully mature. Many annual and perennial plant species produce fruits on indeterminate inflorescences. These flowering stems can have fully ripened seeds within the first to develop basal fruits yet still be flowering at the tip.

Fruit type
Seeds are borne in a variety of fruit types. Fruits are generally classified as dry or fleshy, dehiscent or indehiscent. They may contain a single seed or thousands of seeds per fruit. Seeds may be shed from the parent plant over a short period of time, sometimes explosively, or remain on the plant for considerable periods. Becoming familiar with the various types of fruits and their methods of dispersal will assist the seed collector in making successful collections.

Climate
Timing of rainfall and temperature that affects plant growth also affects seed production. Sudden heat spells or prolonged cool periods can also greatly hasten or delay fruit ripening and dispersal.

Elevation
If seeds have already dehisced or are still immature, search for ripe seed from populations at higher or lower elevations.

Micro-habitats
Warmer, cooler, drier or more mesic sites within a plant population influences seed availability. For example, if the seed has dispersed from most of the plants in the primary population, plants on a more northern exposure or in a swale may still have seed available.

Fully ripened seed may not always be warranted or necessary. Although storage life is compromised, immature seed of some species may germinate more reliably. This is because by harvesting seed early, the development of dormancy mechanisms in the seed is interrupted.

Exceptions: There are some situations where immature fruits will have fully matured seeds that are dark in color and having separated from the fruit wall are loose in the pod. Slightly immature fruits for some plant species can be harvested and if kept under moderate temperatures and humidities will mature and dehisce into the collection bag. Cut flowering stems with slightly immature fruits can be placed into water to continue ripening. Harvested flowering inflorescences of Dudleya species, wrapped in newspaper and kept under room conditions, can self-pollinate, develop fruits and produce fertile seeds.

How - making high quality collections

Impacts & Ethics
"The Earth's biotic communities are an endowment for humanity. The challenge facing the human species is to avoid depleting the principal at the expense of the interest." (Paraphrased from a talk by Dr. Peter Raven, Director of Missouri Botanic Garden.

When making seed collections - first do no harm. Evaluate the population size and the current season's fruit production. Can the population size and current year's seed output tolerate seed sampling and, if so, at what level? Is it possible that others may also be collecting seed from plants in the same region? It is generally best to avoid and it is sometimes illegal to collect along roadsides and in regularly visited public areas. Roadside areas however are often the only sites where a given plant species occurs in the region and they are often most abundant in these open and frequently more mesic areas. When collecting along roadsides it is best to first be safe and to also be discreet in collecting.

If a plant species is very rare (in nature or in the local area), if the population size is small or if seed production is limited, seed collections should only be undertaken on a very limited level - if at all. In these cases consider whether a smaller collection and multi year collection strategy, or if horticultural seed regeneration would be more appropriate?

In *Ex-situ Plant Conservation Supporting Species Survival in the Wild*, 2004, in the chapter "Effects of Seed Collection on Extinction Risk of Perennial Plants", Menges, Guerrant and Hamzé present three seed harvest rules:

- Harvesting 10 percent of the seeds in 10 percent of years (every ten years or less) is generally safe.
- Harvesting 50 percent of seeds in 50 percent of years (every other year or more) is generally unsafe.
- Less intense, frequent harvests are safer than more intense, infrequent harvests.

Most state and federal guidelines limit sampling to no more than 5% of the current season seed either on a population or on a per-plant basis. Where a plant population or portion of a population is to be extirpated, collecting up to 100% of the seeds as well as taking cuttings or whole plants from the affected portion of the population is obviously appropriate.

Sampling methods and techniques

One is likely to capture a higher percentage of a population's genetic diversity by collecting fewer seeds from more individuals as opposed to collecting more seeds from fewer plants. Again, depending upon the use of the seed collection, one may or may not be concerned with the genetic diversity of a seed collection. However, for most situations the more individuals contributing to a seed collection, the more useful it is. General guidelines to capture 95% of the genetic diversity of a plant population call for sampling seed from a minimum of 30 individuals selected randomly and evenly from throughout a population. (For conservation collections current CPC guidelines recommend sampling 50 individuals) Genetic diversity is generally higher for out-crossing species and lower for self-pollinating species. Thus populations of self pollinating species should be sampled at a higher rate.

Depending on the purpose of the collection it may be especially important to seek out and sample populations or individuals that are growing in unique habitat conditions as these individuals are more likely to vary genetically. "This is because where a plant grows (its habitat) is a better indicator of its genetic variation than its appearance or phenotype." (Brown & Briggs 1991)

Depending upon the purpose and ultimate use of the seed collection, collections can be made consisting of seed from all individuals sampled and packaged collectively (***bulk collections***), or the collection can be made where seeds from each parent plant sampled is kept separate (***maternal line collections***). Where collections are to be used for seed regeneration, storing seed along maternal lines will enable the horticulturist, restorationist, or land steward to determine the number of parental individuals contributing to the regenerated seed collection. This process allows for the greatest chance of reintroducing the genetic diversity inherent in the original population and thereby increasing the chances for the introduced or augmented plant population to adapt and thrive.

Take the time to assess the distribution of the target plant species and estimate the number of individuals in the population. As is practical, sampling should be done randomly and evenly from throughout the population.

It is typical for different individuals within a population to produce more seeds than others. When sampling seeds for conservation or restoration purposes it is important to collect a fairly uniform quantity of seed from each sampled individual and not to bias a "bulk" seed collection in favor of a few unusually productive individuals.

Collection Methods
In general, dry fruits are harvested into paper envelopes or bags while moist fruits are collected in plastic bags, buckets, etc. Fruits can be hand plucked or knocked from the parent plant, whole plants can be harvested (for those diminutive ephemeral annual species), inflorescences with ripe or ripening seed can be cut and placed into collection bags, or ripe seed can be shaken directly into baskets, sheeting, or bags placed under the plant. Cloth bags can be securely placed around ripening fruits to catch the seed during their natural dispersal period. This method is helpful for those species that dehisce their seed over a very short period and allows for the capture of seed that has fully ripened on the parent plant. Cloth bags come in a variety of sizes up to 8" x 12".

Conditions at a collection site change dramatically over a 4-6 week period. As later season plants grow over what once were the dominant (and frequently the most spectacular) plants in the area, relocating them later in the season can be quite a challenge. When the plants are in bloom and voucher specimens are being made, it is good practice to note and record fairly specific information as to aspect and slope, micro-habitat, unique geologic features on the site, and plant associations. Placing environmentally and visually benign markers at the population perimeters, as long as one is certain to find and remove them, can also help. The best method of all is having good global positioning system (GPS) coordinates, a topographic map with the population defined, detailed field notes, and patience.

Minimal chaff: While making a seed collection a little extra effort made in the field to minimize the amount of excess plant material gathered makes cleaning easier, improves the drying process, and reduces the likelihood of mold, pathogen, insect or pest plant species contamination.

Post harvest care of collections
Proper care of collections after harvest is important. Moist fruits kept in plastic bags in warm conditions will within a few days ferment and mold. Good air circulation is important even for dry fruits with a high moisture content at harvest time. Harvested material should be placed loosely and not packed into collection bags.

Most moist fruits are easiest to clean shortly after harvest while they are moist. Care should be taken to keep them hydrated and as with any fresh fruit they can be maintained for extended periods under refrigeration.

Dry fruits should be maintained under moderate to warm temperatures and low relative humidity. Many plant species' fruits will open and dehisce their seed into the collection bag during the post harvest period. Harvested plant material can also be loosely wrapped in newspaper, spread out on paper or weed mat covered benches, or over wire mesh sheeting to continue drying. Some fruits release their seed explosively; therefore their collection bags should be well-sealed and spread collections covered. Post harvest care of collections includes protecting them from rodents and keeping them under moderate to cool temperatures until they can be cleaned, safely packaged and placed into storage.

Appendices 1

Summary Table of Sampling Considerations for Rare, Threatened or Endangered Plants
Adapted from the 2004 Center for Plant Conservation, 2003 Royal Botanic Gardens Kew and the 1997 Australian Network for Plant Conservation Germplasm Collection Guidelines

Sampling Question	Considerations or Inputs
Which species should be collected?	1. Degree of endangerment – locally and throughout its range 2. Taxonomic and phenotypic uniqueness - (endemism) 3. Genetic and reproductive stability of the species 4. Ability to store and cultivate the species 5. Existence and condition of ex-situ collections
How many (and which) populations should be sampled per species?	1. Degree of endangerment or threat to a population 2. Genetic and reproductive stability of a population 3. Range and distribution of the taxon 4. Degree of gene flow among populations. (Mating systems) 5. Unique ecotypes 6. Conspicuous polymorphism between populations
How many (and which) individuals should be sampled? Up to 50* *Benchmark to capture genetic variation. *If seed output is low or when conducting parallel collections for backup storage sampling of more than 50 individuals may be required*	1. Local abundance 2. Eminent threat(s) to survival of a population 3. Genetic and reproductive stability of the species (seedling establishment, plant vigor and recruitment success) 4. Species method(s) of reproduction, seed (sexual) or vegetative (clonal) 5. Seed viability and production 6. Anticipated splitting of collections for secondary parallel collections - (double number of samples) 7. Conspicuous eco-typical variation within a population habitat or microsite 8. Conspicuous polymorphism within populations 9. Mating systems: self pollinating (up to 50), obligate out-crossers and mixed mating systems (30-50)
How many (and which type of) propagules should be collected? Target quantity of 2500 “viable “ seeds without taking more than 10% of seed produced in 10% of the years - or - between 2 - 5% annually in a multiyear effort Cuttings: between 1 - 10 per individual	1. Seed type (orthodox or recalcitrant) 2. Appropriate facilities to store and/or cultivate the species 3. Availability of seed or vegetative material 4. Seed viability, seed predation, seed germination rate 5. Anticipated success rate in rooting cuttings 6. Storage tolerance of seed collections or survival of plants in cultivation 7. Anticipated splitting of collections for secondary parallel collections - (double number of samples) 8. Long-term use of the collection (anticipated attrition for: viability testing, research, reintroduction attempts)
Under what circumstances is a multi-year collection plan indicated?	1. To compensate for low numbers of individuals in a population; inadequate annual seed or vegetative output; low seed germination rates; demonstrated poor seedling development due to inbreeding depression or other genetic factors 2. To increase genetic diversity in a collection by repeat sampling over a period of years 3. To augment limited or declining ex-situ collections

Appendices 2

Collecting Permit Contacts

Permitting Agency	Contact	Phone
Angeles NF	Bill Brown - Forest Biologist	(626) 574-5258
Cleveland NF	Kirsten Winter - Forest Biologist	(619) 673-6180 x 3056
El Dorado NF	Mike Taylor - Botanist	(530) 621-5219
Inyo NF	Kathleen Nelson OR Sue Weis - Botanists	(760) 873-2498
Klamath NF	Susan Stresser - Wildlife Biologist	(530) 841-4538
Lassen NF	Allison Sanger - Forest Botanist	(530) 252-6662
Los Padres NF (MOU)	Mike Foster - Forest Botanist	(661) 245-3731
Mendocino NF		(530) 934-3316
Modoc NF	Jim Irvin - Forest Ecosystem Staff Officer	(530) 233-8836
Plumas NF		(530) 283-2050
Rogue River NF	Wayne Rolle - Forest Botanist	(541) 858-2274
San Bernardino NF	Melody Lardner - Forest Botanist	(909) 382-2725
Sequoia NF	Fletcher Linton, Forest Botanist	(559) 784-1500 x 1185
Shasta-Trinity NF	Julie Nelson - Forest Botanist	(530) 226-2429
Sierra NF	Joanna Clines - Forest Botanist	(559) 877-2218 x 3150
Siskiyou NF		(541) 858-2200
Six Rivers NF	John McRae - Forest Botanist	(707) 441-3513
Stanislaus NF		(209) 532-3671
Tahoe NF	Blaze Baker - Forest Botanist	(530) 265-4531
Toiyabe NF (Nev)	Joanne Baggs - Forest Botanist	(775) 331-5341
Bureau of Land Management	John Willoughby - State Botanist	(916) 978-4638
USFWS - TE-009018-2	Jim Bartel, Carlsbad FWS office	(760) 431-9440
	Connie Rutherford, Ventura FWS Office	(805) 644-1766 x 306
	Ken Fuller or Diane Elam, Sacramento FWS Office	(916) 979-2120
California Department of Fish and Game M.O.U. State Listed Species	Mary Ann Showers Sandra Morey, Sacramento office	(916) 327-5222
Catalina Conservancy	Peter Schuyler	(310) 510-1299
San Clemente Island - USFWS	Kim O'Conner	(619) 545-3488
National Forest Sensitive Species	Anne Bradley	(415) 705-2691

Helpful government agency websites

http://www.fs.fed.us/fs/directories/
http://www.fs.fed.us/r5/forest-offices.html
http://www.ca.blm.gov/directory.html
http://permits.fws.gov/
http://www.cites.org/
http://international.fws.gov/cites/cites.html