SEED COLLECTION GUIDELINES

For California Native Plant Species

Michael Wall, Seed Conservation Program Manager ~ Rancho Santa Ana Botanic Garden

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 in seed banks provide material for educational display, horticulture, research, and rare plant reintroduction and population enhancement programs.

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

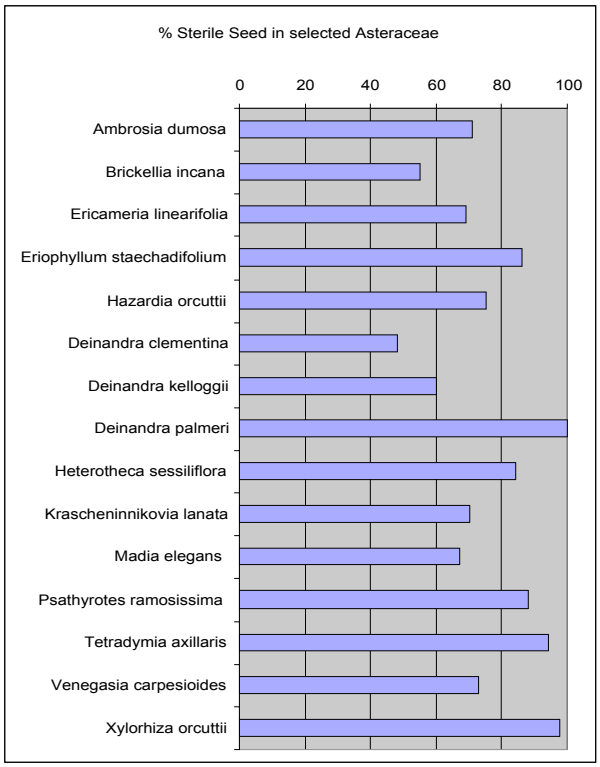
Quality should always trump quantity. Making high quality seed collections is the primary goal for any seed collector.

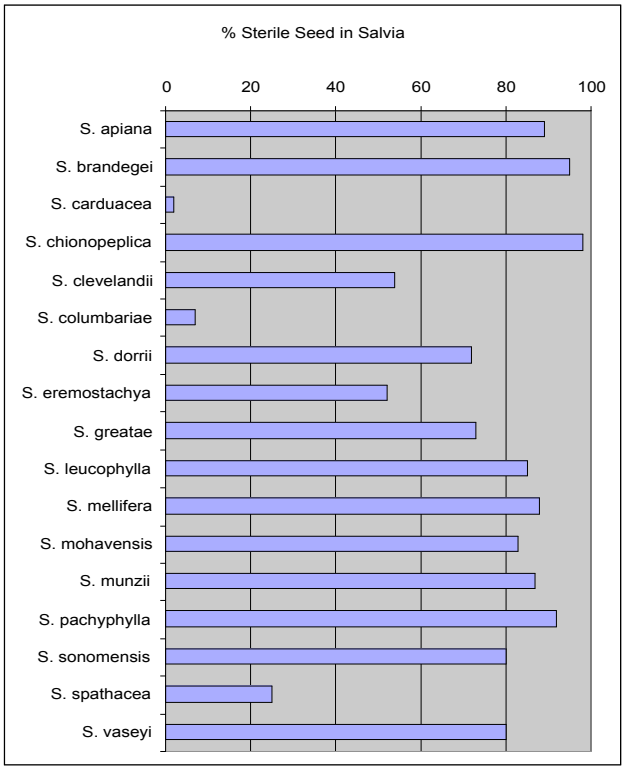
Correct species identification and making voucher herbarium specimens
The American Heritage Dictionary defines voucher as a verb "to substantiate or authenticate with evidence". Properly collected and documented herbarium specimens provide the evidence necessary to verify a species identity. Vouchers 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*.

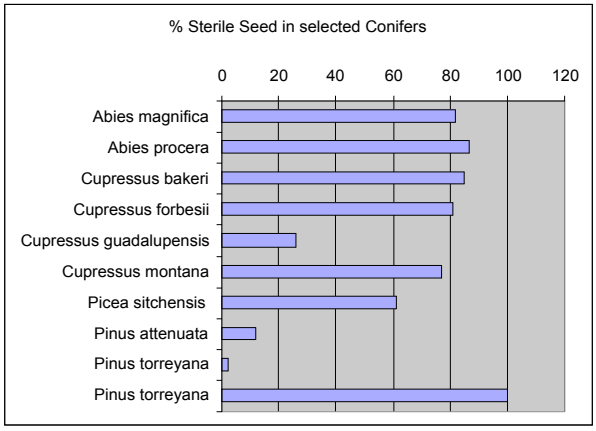
A voucher specimen is an essential component of a high quality seed collection. Typically voucher specimens are collected on scouting trips during the flowering season followed by a return trip or sometimes multiple trips to a site when fruits and seeds have ripened. If flowering voucher specimens are 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.

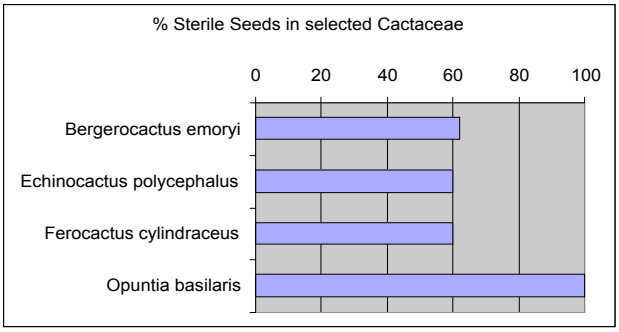
Healthy, sound, viable seed
Maximum germination, seedling vigor, and longest storage life is achieved when fully ripened, current season, pest-free seed is harvested. Environmental conditions, lack of pollinators, and parasitism all can affect the quality of the seeds collected. It is important to note 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. There can be a high percentage of aborted or hollow/sterile seeds in any seed lot. Noting the percent viability of a given seed lot allows for better seed collections and control of results during propagation.

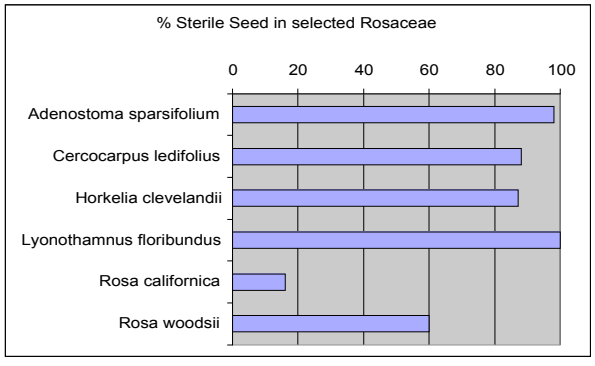
The graphs on this page represent seed lots of RSA collections where seed abortion rates have been documented at RSABG. Percent viable seed varies from one seed lot to the next, among families and genera. Plants that produce small seeds, and most annual plant species, tend to produce a higher percentage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

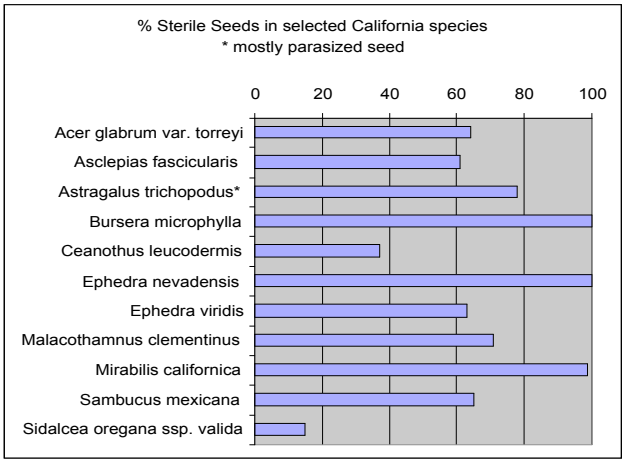












Viability assessments and considerations in the field
Two of the most common methods for estimating viability and seed quality are floatation and dissection. Making a "cut test" dissection exam to check seed development and soundness can easily be done in the field using a single edge razor, a small 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 desiccation 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:
Collection date
Species identity (as can be determined in the field)
Descriptive and precise locality information including habitat and associated species
Site conditions including sun / shade exposure, soil type, aspect, degree of slope, and elevation
Local abundance, population size and number of individuals sampled
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.

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.

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.

Many 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.

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.

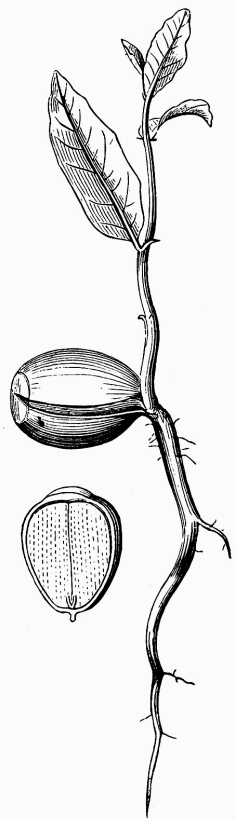
SEED STORAGE GUIDELINES

For California Native Plant Species

Michael Wall, Seed Conservation Program Manager ~ Rancho Santa Ana Botanic Garden

The five most important factors affecting seed longevity are:

1. Seed type
2. Seed quality
3. Integrity of the protective seed coat
4. Seed moisture content
5. Storage environment



Inside each seed is a living plant embryo that even in a state of dormancy breathes through the exchange of gases and is constantly undergoing metabolic (aging) processes. The natural lifespan of a seed is influenced by several factors including: permeability of the seed coat, dormancy, seed physiology, and the storage environment. Seeds of many of our native plants and weedy alien species have dormant embryos and hard seed coats, a condition that retards germination and consequently enhances longevity. The presence and degree of seed dormancy and subsequent metabolic rate varies considerably between species and thus influences their natural lifespan. For most species from temperate and arid climates reducing and maintaining a low seed moisture content, storing seeds at moderate to low temperatures, and taking precautions not to damage seeds during cleaning and handling, slows down the metabolic process and thereby increases their longevity in storage.

Seeds are generally categorized into the following types: [1]

- **Orthodox.** Seeds that can be dried, without damage, to low moisture contents, usually much lower than those they would normally achieve in nature. Their longevity increases with reductions in both moisture content and temperature over a wide range of storage environments.
- **Recalcitrant.** Seeds that do not survive drying to any large degree, and are thus not amenable to long term storage.
- **Intermediate.** Seeds that are more tolerant of desiccation than recalcitrants, though that tolerance is much more limited than is the case with orthodox seeds, and they generally lose viability more rapidly at low temperature.

Recalcitrant seeds are not only desiccation-sensitive, but also metabolically active. In contrast, orthodox seeds, owing to their dry state, are metabolically quiescent.[2]

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1 1 RBG KEW Seed information Database, http://www.kew.org/data/sid/storage.html

2 Science 7 January 2005: Vol. 307. no. 5706, pp. 47 – 49 Patricia Berjak, Protector of the Seeds: Seminal Reflections from Southern Africa

One can estimate a species' natural potential for storage tolerance by:

Seed size. Large seeds often have a high moisture or oil content and are generally recalcitrant in their storage behavior.

Seed physiology. A heavy impervious seed coat even on large seeds, as is often found on desert legumes and lupines, promotes long-term seed viability.

Climate and habitat conditions in which the species grow. Seeds from plants adapted to tropical or riparian habitats, due to a semi to permanent water source and/or consistently mild and reliable growing conditions, may not require long term seed viability for survival. Conversely, plants from desert, temperate and Mediterranean climates, where environmental conditions suitable for germination are often infrequent, are more likely to produce seeds capable of surviving for long periods.

Life cycle. Annuals and perennials are more dependent on a persistent soil seed bank than woody and long-lived shrub and tree species.

Ecological associations. Plants that are early successional colonizer species that may occur only after a disturbance and species that depend on other plants for their development must maintain viability until a suitable host plant is available.

SEED LONGEVITY IN THE NATURAL ENVIRONMENT

For many plant species a substantial portion of their population exists below the soil surface in the form of a soil seed bank. Under the right conditions seeds of many plant species can survive for extremely long periods. The following examples of extreme longevity illustrate the potential for long term seed viability.

In 1966, seeds of the Arctic lupine *Lupinus arctica* were recovered from a rodent burrow six meters below the frozen silt surface and later successfully germinated. Along with the seeds a collared lemming skull was found in the burrow. Since this lemming species disappeared from the region 10,000 years ago it was proposed that the seeds were also this age. Plants of the sacred lotus *Nelumbium nuciferum* were grown from seeds whose fruits were carbon dated at more than 1,200 years old. These seeds were discovered in an ancient peat bed in Pulantien, Liaoning Province, China. In Denmark , Odum (1965) extracted and germinated seeds of *Chenopodium album* and *Spergula arvensis* from soil samples collected beneath buildings. These seeds were estimated by the dates of the structures to be over 1700 years old.[3] In 2005 2,000 year old seeds of the Judea date palm *Phoenix dactylifera* were germinated and produced healthy plants. These seeds were discovered in 1970 in an archeological dig in Israel. After the discovery the seeds were stored away in a desk and there remained until they were successfully germinated. The age of the seeds was determined by carbon dating of the seeds from the same lot that produced the plant.[4] In 2006 seeds were germinated and healthy plants produced from 200 year old seeds of an

3 Seeds: Ecology, Biogeography, and Evolution of Dormancy and Germination. Carol C. & Jerry M. Baskin, pages 145-148, 1998

4 BBC News, Monday, 13 June, 2005, 01:21 GMT 02:21 UK, http://news.bbc.co.uk

Acacia species that were discovered in a merchant's notebook that had been stored since 1803 in the Tower of London.[5]

While these seed longevities are interesting, they are the exception rather than the rule. Under most circumstances even the hardiest of seeds that are shed into their natural environment face a host of hazards including degradation from exposure to harsh environmental conditions, predation, and the chance distribution of ending up in places wholly inappropriate for germination and establishment.

SEED LONGEVITY UNDER CONTROLLED CONDITIONS

Seed banking, the practice of maintaining seeds under safer and more controlled conditions, has proven to be extremely effective in slowing down the aging process thereby maintaining viability and seed vigor over extended periods.

The practice of seed preservation is as old as agricultural practices but systematic collections and storage facilities have been a development of the 20th century. Today there are an estimated 1300 seed or gene banks around the world containing over 6 million seed accessions. Seed banking from a species conservation perspective has many benefits as well as some negative consequences. Establishing a seed storage program, i.e. maintaining viability in seed lots so that they will be available when needed, is generally more cost effective than making field collections for annual propagation needs. Seed production in nature is frequently unreliable and adequate seed may not be available in the year that it is needed. Having a system in place to properly handle and store seeds allows one to take advantage of good crop years. Especially for annual and some perennial species, propagating from seed lots made over multiple years from one population increases the genetic diversity represented in the plants and any subsequent regenerated seed. Maintaining germplasm in seed banks serves as a hedge against extinction for threatened wild plant species, their populations, or cultivated landraces of agricultural plants.

For some plant species, using relatively fresh rather (that which is less than one year old) gives superior germination over stored seed. Viability of a seed lot declines over time and though old seed may germinate, the resulting seedlings may have reduced vigor and fail to establish as well as seedlings from fresh seed. While seed storage takes up relatively little space, an adequate seed storage program requires considerable time, materials and energy. Additionally, horticultural selection during propagation events can potentially have negative effects on the genetic make up of regenerated seed lots. Finally, conserving plants in ex-situ seed banks over long periods of time removes them from natural selection processes. In the case of annuals and short-lived perennials, it may be questioned whether the plants that result from seeds stored in long-term storage collections (e.g. greater than 30 years) are really the same entity once they are grown out and reintroduced into the wild.

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5 http://news.bbc.co.uk Monday, 13 June, 2005, 01:21 GMT 02:21 UK

Principles of seed storage

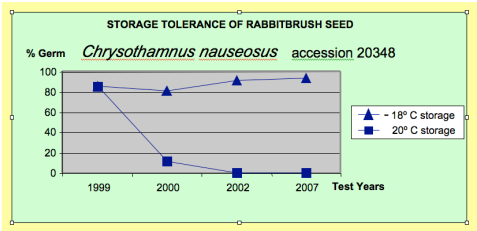
In the natural environment and when stored at ambient room conditions, seeds respond to constantly changing relative humidity and temperatures. Maintaining seeds under controlled conditions lowers metabolic activity, thereby reducing the aging process and increasing longevity of the seed lot. For most seeds, a cool and dry environment is preferred and for orthodox seeds the cooler and drier the greater the longevity that can be achieved. Harrington's rule[6] states that:

1. Each 1 percent reduction in moisture content doubles the life of the seed.
2. Each 10 degree F reduction in temperature doubles the life of the seed.

Rabbitbrush (*Chrysothamnus nauseosus*.) is an example of one plant species whose seeds lose viability rapidly[7] but whose viability can be greatly extended under controlled conditions in an ex-situ seed bank.[8]

Rabbitbrush and California buckwheat
Autumn in the San Bernardino National Forest

Photo
Michael Gordon Photography



Results of viability tests on rabbitbrush seed cold storage vs. room storage over an 8-year period

Orthodox seeds can be dried down to very low moisture content (1-14 percent) and frozen. There is some current discussion as to the optimum storage seed moisture content. The FAO/IPGRI Genebank Standards (1994) recommends drying seeds to low moisture contents (3–7% fresh weight, depending on the species) and storing them in hermetically-sealed containers at low temperature, preferably -18˚C or cooler. This is achieved by drying seeds to equilibrium at 10–15% RH and a temperature of 10°–25°C.[9] There is some concern, however, that drying seeds to these levels, combined with storage at very low temperatures, can be damaging for some species and can actually shorten storage life.[10] According to Dr. Christina Walters at the National Center for Genetic Resource Preservation (NCGRP), the optimum seed moisture content should be based on the storage temperature and her current recommendations suggest that the optimum water content for seed storage increases with decreasing storage temperatures (i.e. the greater the temperature difference between the drying and storage temperatures, the higher the allowable RH

6 "Harrington's Rule" Principles and Practices of Seed Storage, U.S. Department of Agriculture, 1978

7 Monsen and Stevens 1987

8 M. Wall 2007; Comparative germination study on room stored vs. frozen Chrysothamnus nauseosus seed; RSABG, unpublished data

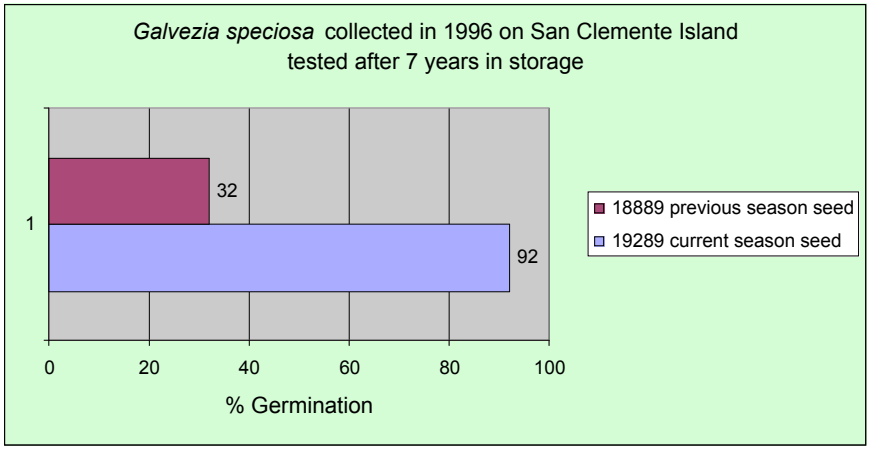
9 Rao NK, Hanson J, Dulloo ME, Ghosh K, Nowell D and Larinde M. 2006. Handbooks for Genebanks No. 8. Rome, Italy

10 Christina Walters, 2004, Appendix 2 in Ex Situ Conservation Supporting Species Survival in the Wild. Island Press

for drying).[11] The following chart from the referenced publication shows the recommended drying conditions for orthodox seeds stored in moisture proof containers.

Drying Temperature (°C)	Drying RH for Storage at 15° C	Drying RH for Storage at 5° C	Drying RH for Storage at -18° C
25	28	33	46
15	20	26	38
5	14	20	32

In general, the sooner orthodox seeds are dried down and placed into storage the greater their longevity. Seeds harvested prematurely may have shorter shelf lives than seeds harvested when fully mature. This may be because immature embryos are not fully tolerant of desiccation and so are damaged when dried. Seeds harvested at an immature stage of development should be dried slowly, preferably within their fruiting structures and at higher humidity than matured seed collections.[12] Old seeds from previous season's fruits can also have a shorter storage life and/or viability. The following germination test results demonstrate storage tolerance variation on previous season (collected in March) and current season (August collection) seed from two separate San Clemente Island populations.



Recalcitrant seeds of temperate species (oaks and walnuts) can be maintained from between 0.5 and 2 years when kept moist and at a temperature just below the minimum temperature needed for germination. Seeds can be stored in moist peat, vermiculite, or Sponge Rock. A fungicide is frequently applied to the seeds to control microbial activity.

Intermediate seeds will generally not tolerate freezing and therefore should be stored at 5° C or above. Because they are more tolerant of drying than recalcitrant species, they can be dried down to lower RH values (40 - 60 percent) which will increase storage life. Moisture can be controlled with desiccants and/or with fungicides. For all storage situations seeds should not be exposed to light.

11 Christina Walters, 2004, Appendix 2 in Ex Situ Conservation Supporting Species Survival in the Wild. Island Press

12 Christina Walters, 2004, Appendix 2 in Ex Situ Conservation Supporting Species Survival in the Wild. Island Press

To determine seed type the seeds can be tested prior to storage. Seeds that tolerate desiccation (show no loss in viability) to 5% moisture content or below (values in equilibrium with 10–15% RH at 20°C) are likely to show orthodox seed-storage behavior. Seeds that tolerate desiccation to about 10–12% moisture content (values in equilibrium with 40–50% RH at 20°C), but whose viability is reduced when subjected to further desiccation to a lower moisture content are likely to show intermediate seed storage behavior. Seeds that are killed by desiccation to 15–20% moisture content (values in equilibrium with >70% RH at 20°C) are likely to be recalcitrant. Information on storage behavior of a wide range of species is available at *www.rbgkew.org.uk/data/sid*.

SEED STORAGE AT RANCHO SANTA ANA BOTANIC GARDEN

The oldest seed collections from the Garden are currently at the National Center for Genetic Resource Preservation as part of the Fritz Went and Philip Munz long term seed longevity experiment.[13] Seeds of more than 100 different native species from a wide range of families and habitats were supplied by the Garden for Dr. Went's 1947 experiment. The seeds were dried down to very low moisture content and placed into glass tubes that were then sealed under vacuum. The seeds were divided into 20 sets with the experiment intended to run for 360 years or until 2307. In 1997, as part of her Master's Thesis, Teri Christensen conducted the fifty year viability testing.[14] Some of the results of her review of the collection are presented here in Appendix 1.

When the Garden moved to Claremont in 1951 the Garden's seed collections were stored in glass mason jars and housed in the stone building at the bottom of the east slope of the Mesa. In 1988, to extend the longevity of the Garden's seed collections, an upright refrigeration unit and a chest style freezer were purchased and installed in the stone house. At this point the horticultural seed collections were transferred from the glass mason jars into double sealed plastic storage bottles and placed into the refrigerator at 5 º C / 41 º F. The endangered species collections following 2 to 3 weeks storage at 12% relative humidity were packaged and sealed in Crystal Springs© storage pouches and placed into chest style freezers at -18 º C / 0 º F. It was customary during this period for seed collections of rare but non-listed plant species to be split between the Horticulture collections and the Endangered Species Program collections. In 1995, both the cold storage and the freezer units were moved into the new Fletcher Jones Seed Storage facility. In 1998 additional chest style freezers were installed and the horticultural collections were dried to equilibrium at 12% RH over calcium sulfate desiccant and placed into storage at -18º C. The rare, threatened and endangered species collections were re-dried and transferred from the "Crystal Springs" storage pouches to the newer and sturdier Barrier Foil© laminate seed storage pouches. Thus over this time some of the oldest seed collections have been stored at a wide range of temperatures from room temperature (16º - 27 º C), refrigerated (5º C) to frozen (-18º C).

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13 F.W. Went and P.A. Munz, April 27, 1947. A Long Term Test of Seed Longevity, El Aliso; Vol. 2, No. 1.

14 Teri Christensen, May 2000, Germination of 91 Native Species after 50 Years in Vacuum Storage, University of Northern Colorado,

Appendix 1: 50 year test results on selected species from the Went and Munz Long Term Test of Seed Longevity for seeds stored in vacuum

		Test Year ~ % Germ		
Family	Went and Munz Test Species	1947	1967	1997
Asteraceae	Achillea millefolium (borealis)	98	98	62
Chenopodeaceae	Allenrolfea occidentalis	5	40	60
Chenopodeaceae	Atriplex hymenolytra	0	10	20
Philadelphaceae	Carpenteria californica	100	35	32
Fabaceae	Cercidium microphyllum	40	56	97
Asteraceae	Chaenactis glabriuscula var. glabriuscula	33	32	30
Polygonaceae	Chorizanthe staticoides	22	22	36
Asteraceae	Cirsium occidentale	77	68	90
Onagraceae	Clarkia (Godetia) amoena var. lindleyi	85	89	88
Onagraceae	Clarkia (Godetia) amoena	96	92	68
Onagraceae	Clarkia (Godetia) bottae	nd	90	82
Onagraceae	Clarkia (Godetia) cylindrica	90	80	56
Onagraceae	Clarkia (Godetia) dudleyana	90	96	100
Onagraceae	Clarkia elegans	100	95	88
Onagraceae	Clarkia (Godetia viminea) purpurea ssp. viminea	92	90	36
Asteraceae	Coreopsis bigelovii	58	63	8
Asteraceae	Coreopsis maritima	nd	93	86
Crossosomataceae	Crossosoma californicum	72	37	44
Asteraceae	Encelia actoni	13	7	0
Polygonaceae	Eriogonum arborescens	7	59	58
Asteraceae	Eriophyllum lanatum	nd	22	20
Papaveraceae	Eschscholzia caespitosa	55	85	34
Papaveraceae	Eschscholzia californica	78	75	0*
Asteraceae	Geraea canescens	7	3	12
Polemoniaceae	Gilia achilleifolia	92	78	38
Polemoniaceae	Gilia chamissonis	72	94	92
Polemoniaceae	Gilia tricolor	96	94	0*
Asteraceae	Lasthenia (Baeria) maritima	49	90	54
Asteraceae	Lasthenia glabrata	36	70	0
Asteraceae	Layia platyglossa	20	14	6
Polemoniaceae	Linanthus grandiflorus	92	97	6
Polemoniaceae	Linanthus montanus	80	94	0*
Fabaceae	Lotus scoparius var. scoparius	7	0	10
Fabaceae	Lupinus succulentus	13	38	24
Asteraceae	Malacothrix arachnoidea	25	29	16
Loasaceae	Mentzelia lindleyi	4	62	10
Lamiaceae	Monardella lanceolata	95	96	94
Hydrophyllaceae	Nemophila maculata	30	80	90
Onagraceae	Oenothera deltoides	52	91	52
Scrophulariaceae	Penstemon heterophyllus	nd	54	82
Scrophulariaceae	Penstemon spectabilis	12	50	0*
Hydrophyllaceae	Phacelia ciliata	43	99	30
Hydrophyllaceae	Phacelia parryi	82	99	30
Hydrophyllaceae	Phacelia tanacetifolia	23	76	52
Hydrophyllaceae	Phacelia viscida	55	97	80
Lamiaceae	Prunella vulgaris (Brunella)	nd	98	94
Platanaceae	Platanus racemosa	45	63	4
Lamiaceae	Salvia columbariae	nd	64	0*
Iridaceae	Sisyrinchium bellum	23	49	0*
An * indicates that no vacuum existed ~ nd = no data				

Appendix 2: Seed germination test results from selected RSA collections. Number of years in storage represents the germination test year minus the store date. Shaded groupings indicate a tread over time in storage; increasing, decreasing or stable.

Accession Num	Lot & Test Num	NAME	Store Date	Years in Storage	%GERM
15405	781*1	Aquilegia formosa	1986	12	66
15405	781*2	Aquilegia formosa	1986	21	52
14262	847*4	Berberis haematocarpa	1977	25	72
14529	886*1	Carex spissa	1981	26	42
14620	1632*1	Carpenteria californica	1980	10	8
14620	1632*3	Carpenteria californica	1980	12	49
14620	1632*6	Carpenteria californica	1980	23	66
14702	901*1	Ceanothus cuneatus	1983	23	31
15875	1641*1	Clarkia amoena ssp. huntiana	1987	2	47
15875	1641*2	Clarkia amoena ssp. huntiana	1987	20	57
15877	1643*1	Clarkia arcuata	1987	2	15
15877	1643*2	Clarkia arcuata	1987	20	80
15884	1650*1	Clarkia concinna ssp. concinna	1987	2	8
15884	1650*2	Clarkia concinna ssp. concinna	1987	14	65
15884	1650*3	Clarkia concinna ssp. concinna	1987	16	75
15610	1686*1	Cordylanthus maritimus ssp. maritimus	1987	3	40
15610	1686*2	Cordylanthus maritimus ssp. maritimus	1987	4	61
15610	1686*3	Cordylanthus maritimus ssp. maritimus	1987	20	72
15327	228*1	Coreopsis maritima	1985	16	79
14732	1689*1	Cupressus bakeri	1982	20	54
14732	1689*2	Cupressus bakeri	1982	24	63
15164	1048*1	Dodecatheon clevelandii ssp. insulare	1983	24	94
14216	1703*1	Dudleya nesiotica	1979	12	96
14216	1703*2	Dudleya nesiotica	1979	21	83
14216	1703*3	Dudleya nesiotica	1979	28	96
15207	1094*1	Echinocereus engelmannii	1985	19	32
15593	1098*1	Elymus glaucus	1987	18	38
15105	1706*1	Eriastrum densifolium ssp. sanctorum	1986	5	82
15105	1706*2	Eriastrum densifolium ssp. sanctorum	1986	14	79
15105	1706*3	Eriastrum densifolium ssp. sanctorum	1986	21	66
15358	229*1	Eriogonum giganteum var. giganteum	1983	14	54
15358	229*2	Eriogonum giganteum var. giganteum	1983	22	43
15358	229*3	Eriophyllum staechadifolium	1987	15	60
19262	2182*1	Ferocactus viridescens	1984	23	60
15292	1186*1	Fraxinus velutina	1984	23	0
14951	1291*1	Hyptis emoryi	1983	24	63
19219	1624*1	Lupinus excubitus var. austromontanus	1981	25	82
14665	1398*1	Lupinus longifolius	1982	25	100
14936	1425*1	Malosma laurina	1983	15	64
14936	1425*2	Malosma laurina	1983	24	68
14552	1918*4	Mimulus aridus	1981	18	47
14552	1918*5	Mimulus aridus	1981	22	53
14940	63*1	Salvia apiana	1983	24	43
14674	1929*1	Solanum wallacei	1982	23	49
14417	1791*1	Sphaeralcea ambigua var. rosacea	1980	27	81

Summary Table of Sampling Considerations for Rare, Threatened, or Endangered Plants

Adapted from Guerrant, Havens and Maunder 2004. Royal Botanic Gardens Kew, 2003.
Australian Network for Plant Conservation Germplasm Collection Guidelines, 1997.

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

Print Form | Submit by Email

Rancho Santa Ana Botanic Garden
California's Native Garden

Accession # _____

Collection Date:				Received Date:							
Species Name:											
Collector Name:						Collection #:					
Associate Collectors:											
Voucher? No		Yes		Location of voucher:							
Country:		State:		County:							
Physiographic region:						Elevation: FT.		M.			
Locality:											
CNDDB EO#:		Landowner:									
Latitude: N		Longitude: W			NAD83		NAD27		WGS84		
Map Quad:			T		R		SEC.		1/4 S.		

Sampled population size: _____		Seed		Division	
Number of individuals sampled: _____		Spore		Cutting	
Locally: common		Plant		Bulb/Corm	
scattered					
rare					

Other: _____

Associated species:

California Floristic Province					
Californian		Sonoran: Mojave		Sierran/Cascade	
Great Basin		Sonoran: Colorado		North Coast Ranges	
				Klamath/Siskiyou	

Habitat:				Slope:		Exposure:		Moisture:	
Alpine		Chaparral		Flat		Full sun		Dry	
Sub Alpine		Scrub		Gentle		Semi shade		Moist	
Forest		Riparian		Steep		Shade		Wet	
Woodland		Type: _____		Cliff				Seasonally Moist	
Grassland		e.g. lodgepole pine forest		Aspect					

Geology:				Soil:			
Gabbro		Shale		Sand		Clay	
Granite		Volcanic		Gravel		Humus	
Limestone		Serpentine		Rock		Alluvium	
Sandstone		Other:		Loam		Other:	

Collector notes and observations:

SEED CLEANING WORKSHEET

Species Name: Sphaeralcea ambigua Acc. # SBNF Project 654

Fruit Type:

☐ Capsule ☐ Follicle ☐ Achene ☐ Nut ☐ Nutlet ☐ Berry ☐ Drupe
☐ Legume ☐ Silique ☐ Silicle ☐ Utricle ☐ Cone ☐ Samara ☒ Schizocarp

☒ Dehiscent ☐ Indehiscent

Avg. # seeds per fruit _____ # fruits examined _____

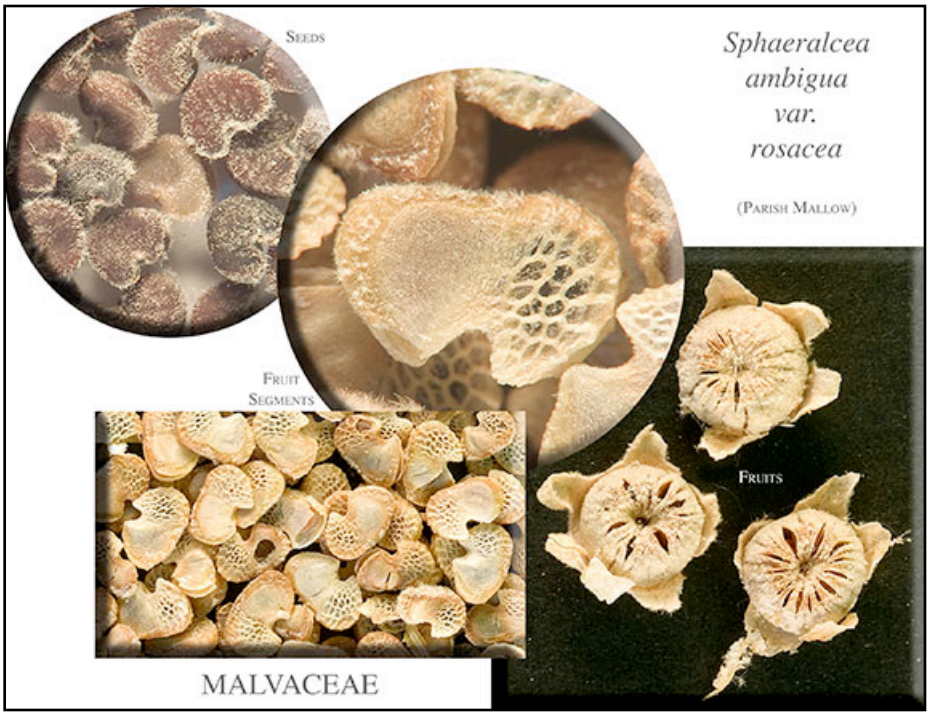
Seed Description: size (mm), shape, color, texture
Fruit: Schizocarp; seed reniform, 1.5mm long by 1.2mm wide, dark brown to tan with short white surface hairs

Cleaning Method:
Dried floral material is placed into a blender with nylon filament line attached to the blades. Blender is filled about half way (ca. 2 cups) and threshed at lowest speed for ca. 1 minute. Sort material through a #12 sieve to separate the threshed seed from the larger floral chaff. Re-sieve 2-3 times. Sort threshed seeds and floral material through a #18 sieve to separate the seed from the finer small chaff. Blower at 30 to sort out chaff and then at 33 to sort out hollow or parasitized seeds.

Post Cleaning Collection Examination Observations:
Percent of seeds with healthy embryos 95 % , # seeds examined 5 of 5 filled at 33 blower speed

Notes:

Difficulty Level: <easy> 1 2 3 4 5 <difficult>



3” x 5” Seed Collection Storage Bottle Index Card Form

Bottle number and seed lot number

Species, collection year, and accession number

Weight of 200 seeds, weight of all seeds and total seeds

Could be used for collection information

Viability information: date checked, number of seeds checked, tissue condition, and method used

S1666 Lot Num 1678

Clarkia williamsonii **1989** **15912**

200 Wt. **0.10g** TWT. **1.472g** TSD. **2,944**

Viability check: **29 Jul 2009, 5 of 5 seeds filled and moist within at 31 blower speed**

S1666 Lot Num 1280

Hibiscus lasiocarpus **1987** **15619**

200 Wt. **2.392g** TWT. **14.736g** TSD. **1,182**

Viability check: **14 Apr 2009 – 5 of 5 floaters partially filled with healthy tissue; 5 of 5 sinkers all filled**

S1666 Lot Num 1188

Fritillaria atropurpurea **1991** **17041**

200 Wt. **0.492g** TWT. **1.026g** TSD. **417**

Viability check: **17 Feb 2009, 5 of 5 seeds filled and moist within at 16 blower speed**

Selected References for Collecting, Processing, Storing, and Germinating Seeds for Conservation and Restoration

Rare plant management, collection and sampling, and reintroduction guidelines

Ex Situ Conservation Supporting Species Survival in the Wild. Guerrant, Havens and Maunder 2004, Island Press

Genetics and Conservation of Rare Plants. Falk, D.A. and Holsinger, K.E. 1991, Oxford University Press

A Basic Sampling Strategy: Theory and Practice. Brown and Marshall, pages 75-91 in Collecting Plant Genetic Diversity: Technical Guidelines. 1995, CAB International for IPGRI, Rome.

Guidelines for the Translocation of Threatened Plants in Australia. 1997 Australian Network for Plant Conservation

Reintroduction of Plants to the Wild. A Handbook for Botanic Gardens. 1995 Botanic Gardens Conservation International

Seed Storage

Seed Conservation - Turning Science into Practice. Smith, R.D., Dickie, J.B., Linington, S.H.,Pritchard, H.W., Probert, R.J. (eds), 2003 Kew: Royal Botanic Gardens.

The Seed Storage Behavior Compendium. T.D. Hong, S. Linington and R. H. Ellis, Handbook for Genebanks No. 4, Kew: Royal Botanic Gardens, 1998. Hard copy of this and the preceeding title available through Kew Gardens at: http://www.kewbooks.com/asps/ShowDetails.asp?id=32

Seed Information Database (release 7.1, May 2008) Liu, K., Eastwood, R.J., Flynn, S., Turner, R.M., and Stuppy, W.H. 2008. http://www.kew.org/data/sid

Germplasm Conservation Guidelines for Australia. 1997 Australian Network for Plant Conservation

Seed Processing and Propagation

Collecting, Processing, and Germinating Seeds of Wildland Plants. Young & Young, 1986 Timber Press, Portland, Oregon

Seeds of Woody Plants in North America. Young & Young, 1992 Dioscorides Press, Portland, Oregon USA

Seeds: Ecology, Biogeography, and Evolution of Dormancy and Germination. Carol C. & Jerry M. Baskin, 1998 Academic Press, 666 pp. USA

Processing Seeds of California Native Plants for Conservation, Storage, and Restoration. Wall, M. and J. Macdonald. August 2009. Rancho Santa Ana Botanic Garden Occasional Publication. Claremont, CA. 216 pages.

Seed Propagation of Native California Plants. Dara Emery, 1988 Santa Barbara Botanic Garden

Seed Collecting, Processing, and Storage: Useful Contacts and Associations

Center for Plant Conservation (CPC) Missouri Botanic Garden St. Louis MO
http://www.centerforplantconservation.org/

Royal Botanic Gardens Kew Seed Information Database Wakehurst Place UK
http://www.rbgkew.org.uk/data/sid/

Australian Network for Plant Conservation Canberra, Australia
www.anbg.gov.au/anpc/

National Center for Genetic Resources Preservation; USDA Agricultural Research Station (ARS) Fort Collins, CO
NCGRP

Biodiversity International
Biodiversity International - Germplasm Collecting

Association of Official Seed Analysts (AOSA), Lincoln, NE
www.aosaseed.com

Front Range Seed Analysts (FRSA)
www.FRSA.org

International Seed Testing Association (ISTA)
www.seedtest.org

Ransom Seed Laboratory - RSL
Ransom Seed Lab

Supply Sources

Hoffman Manufacturing Co. (Blowers and General Agricultural Seed Operation Supplies) Albany, OR 97321
www.hoffmanmfg.com/

Hydrosorbant Products (Silica Gel Desiccant Products) Ashley Falls, MA
www.dehumidify.com

Markson LabSales (Plastic storage bottles, general lab supplies) P.O. Box 3616 Honolulu, HI
www.markson.com

BioQuip Products (Glassine envelopes, gelatin capsules) 17803 LaSalle Avenue Gardena, CA
www.bioquip.com/

Herbarium Supply Company PO Box 10966 Bozeman, MT 59719
www.herbariumsupply.com